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(AQUATIC DISPOSAL)

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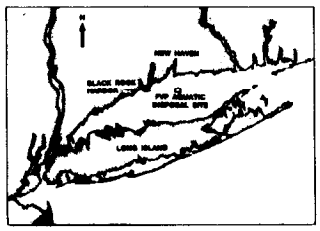
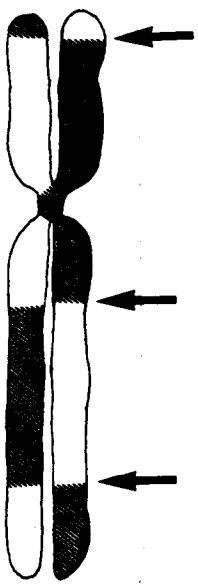
SISTER CHROMATID EXCHANGE IN MARINE POLYCHAETES EXPOSED TO BLACK ROCK HARBOR SEDIMENT





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This report evaluates the use of the cytogenetic technique of sister chromatid exchange (SCE) to measure potential mutagenic activity associated with contaminated dredged material. The three primary objectives were to test the applicability of the SCE technique, to field verify any responses observed in the laboratory, and to determine the degree of correlation between the bioaccumulation of contaminants and the SCE response. This project was part of the US Environmental Protection Agency/Corps of Engineers Field Verification Program (FVP).

The SCE technique was applied to *Nephtys incisa*, an infaunal polychaete dominant in the benthic community at the Central Long Island Sound (CLIS) disposal site. The SCE response was measured in *N. incisa* exposed to suspended and bedded sediment phases of Black Rock Harbor (BRH) dredged material in the laboratory. The experiment was replicated three
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times as a randomized complete block design. The treatment employing suspended BRH sediment over bedded reference sediment was significantly higher than all other treatments.

The SCE response was measured in *N. incisa* sampled along a transect of stations at the CLIS disposal site. The SCE response was low in June 1983 (immediate postdisposal), rose significantly (3.8×) in August 1983, and declined in December 1983. There were no differences among stations on any given date. The laboratory and field SCE data were in excellent agreement. For example, the laboratory controls were statistically the same as the June and December field samples, and the highest laboratory response was statistically the same as the August field data.

BRH sediment contains high concentrations of many contaminants including polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) that accumulate in tissues of worms exposed in both the laboratory and the field. Postdisposal field concentrations of these compounds decreased in surficial sediments with distance from the FVP site. Tissue concentrations of PCBs and PAHs in field-collected *N. incisa* peaked during the summer of 1983 and declined during the fall. Of ten chemicals and two summary statistics analyzed for correlation between SCE response and tissue concentrations, only two, benzo(a)pyrene (BaP) and chromium, are known mutagens. BaP is not a direct-acting mutagen; however, its metabolites are mutagenic. Therefore, a statistical relationship between tissue concentrations of BaP and SCE response would not be expected. Chromium can be a direct-acting mutagen, and a correlation between chromium concentration in tissues and SCE response was found ($r = 0.83$). Because of the multicontaminant nature of the dredged material and the study designs used in the FVP, potential cause-effect relationships between tissue residues and SCE response were not addressed. The increased SCE frequencies associated with exposure to BRH sediment are indicative of mutagenic activity and underscore the need for genotoxicity testing in evaluating wastes to be ocean disposed.

PREFACE

This report describes work performed by the US Environmental Protection Agency (USEPA), Environmental Research Laboratory, Narragansett, R. I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). The FVP was sponsored by the Office, Chief of Engineers (OCE), and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing techniques for predicting the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP was conducted by ERLN, with the wetland and upland portions being conducted by WES.

The principal investigators for this aquatic study and the authors of this report were Dr. Gerald G. Pesch, Ms. Carol E. Pesch, Dr. A. Russell Malcolm, Mr. George R. Gardner, and Dr. Peter F. Rogerson, ERLN; Dr. Wayne R. Munns and Ms. Cornelia Mueller, Science Applications International Corporation (SAIC); and Dr. James Heltshe, Dr. T. C. Lee, and Mr. Andre G. Senecal, University of Rhode Island (URI).

The laboratory exposure system was designed by Dr. Paul Schauer, URI; Mr. John Sewall, SAIC, provided invaluable assistance with all aspects of the laboratory exposure systems. Mr. Michael Balboni and Ms. Ruth Gutjahr-Gobell, SAIC, and Dr. D. Michael Johns, Tetra Tech, assisted with collecting worms and conducting experiments. Data management and analysis were conducted by Mr. Jeffrey Rosen of Computer Sciences Corporation (CSC). The authors wish to thank Dr. John F. Paul, ERLN, for contributions to the field exposure model, and Ms. Joan E. Seites, CSC, for manuscript preparation and extensive word processing support.

Analytical chemistry support was provided by Dr. Gerald Hoffman, Mr. Richard Lapan, Mr. Curtis Norwood, and Mr. Frank Osterman, ERLN; Dr. Richard Pruell, Mr. Richard McKinney, and Ms. Sharon Pavignano, SAIC; and Ms. Kathleen Schweitzer, URI. Dr. James Heltshe, CSC, provided guidance with statistical analysis.

Special thanks are due to Mr. Robert Alix and Dr. Anthony Calabrese of the National Marine Fisheries Service Laboratory, Milford, Conn., for field support and for use of their boat, the SHANG WHEELER.

The USEPA Technical Director for the FVP was Dr. John H. Gentile, ERLN; the Technical Coordinators were Dr. Gerald Pesch and Mr. Walter B. Galloway, ERLN. Technical reviews were provided by WES personnel.

The study was conducted under the direct WES management of Drs. Thomas M. Dillon and Richard K. Peddicord and under the general management of Dr. C. Richard Lee, Chief, Contaminant Mobility and Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison, Chief, Environmental Laboratory. Manager of the Environmental Effects of Dredging Programs was Dr. Robert M. Engler, with Mr. Robert L. Lazor, FVP Coordinator. Dr. Thomas D. Wright was the WES Technical Coordinator for the FVP reports. This report was edited by Ms. Jamie W. Leach of the WES Information Products Division.

The OCE Technical Monitors were Drs. John Hall, Robert J. Pierce, and William L. Klesch. The Water Resources Support Center Technical Monitor was Mr. Charles W. Hummer.

COL Dwayne G. Lee was Commander and Director of WES. Dr. Robert W. Whalin was Technical Director.

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PART I: INTRODUCTION

Background

1. The Marine Protection, Research, and Sanctuaries Act (Public Law 92-532) was passed by Congress in 1972. This law states that it is the policy of the United States to regulate disposal of all types of materials into ocean waters and to prevent or strictly limit disposal of any material that would adversely affect human health, welfare, the marine environment, or ecological systems. The implementation of this law, through the issuance of permits as defined in the final regulations and criteria, is shared jointly by the US Environmental Protection Agency (USEPA) and the US Army Corps of Engineers (CE).

2. In 1977, the CE and the USEPA prepared technical guidance for the implementation of the final ocean dumping regulations in the form of a manual entitled "Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters" (USEPA/CE 1977). This manual specified which test procedures were to be followed in collecting information to be used in making a disposal decision. Among the procedures were those for: (a) chemically characterizing the proposed dredged material; (b) determining the acute toxicity of liquid, suspended particulate, and solid phases; (c) estimating the potential contaminant bioaccumulation; and (d) describing the initial mixing during disposal. These methods have been used for determining the suitability of dredged material for open-water disposal. The procedures in this manual represented the technical state of the art at that time and were never intended to be inflexible methodologies. The recommended test methods were chosen to provide technical information consistent with the criteria specified in the regulations. However, use of the manual in the permit process has identified conceptual and technical limitations with the recommended test methods (Gentile and Scott 1986).

3. To meet this critical need, the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal

Alternatives Program, or the Field Verification Program (FVP), was authorized in 1982. This 6-year program was sponsored by the Office, Chief of Engineers, and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing test methodologies for predicting the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP was conducted by the USEPA Environmental Research Laboratory, Narragansett, R. I. (ERLN). The wetland and upland portions, being conducted by WES, are reported in separate documentation.

4. ERLN was responsible for conducting research on the aquatic portion for disposal of dredged material. There were three research objectives for this portion of the program. The first was to demonstrate the applicability of existing test methods for detecting and measuring the effects of dredged material, and to determine the degree of variability and reproducibility inherent in the testing procedure. This phase of the program (Laboratory Documentation) is complete, and the results have been published in a series of technical reports. This information provides insight into how the various methods function, their sources of variability, their respective and relative sensitivities to the specific dredged material being tested, and the degree of confidence that can be placed on the data derived from the application of the methods.

5. The second objective was to field verify the laboratory responses by measuring the same response under both laboratory and field exposures. A basic and often implicit assumption is that results derived from laboratory test methods are directly applicable in the field. While this assumption is intuitive, there are no supporting data from studies on complex wastes in the marine environment. The study reported herein offers a unique opportunity to test this basic assumption.

6. The third objective was to determine the degree of correlation of tissue residues resulting from bioaccumulation of dredged material contaminants with biological responses from laboratory and field exposure to dredged material. However, this study was not designed to address cause-effect relationships, and the multicontaminant nature of the dredged material precludes any such assumptions.

Project Description

7. The aquatic disposal portion of the FVP was a site- and waste-specific case study that applied the concepts and principles of risk assessment. The disposal site for the FVP is a historical site known as the Central Long Island Sound (CLIS) disposal site (1.8 by 3.7 km) located approximately 15 km southeast of New Haven, Conn. (Figure 1). The sedimentology at the disposal and reference sites is primarily silt-clay, with a mean grain size of 0.013 mm. Thermal stratification occurs from April to September, and during this period bottom salinity is slightly higher than that of the surface. Tidal currents typically dominate the near-bottom water in an east-west direction. Suspended sediment concentrations average 10 mg/ℓ, with storm-induced values to 30 mg/ℓ measured 1 m above the bottom. The baseline community data revealed a homogeneous, mature infaunal community dominated by the polychaete *Nephtys incisa* and the bivalve molluscs *Nucula proxima* and *Yoldia limatula*.

8. The FVP disposal site was selected within the CLIS so as to minimize contamination from other sources, including relic disposal operations or

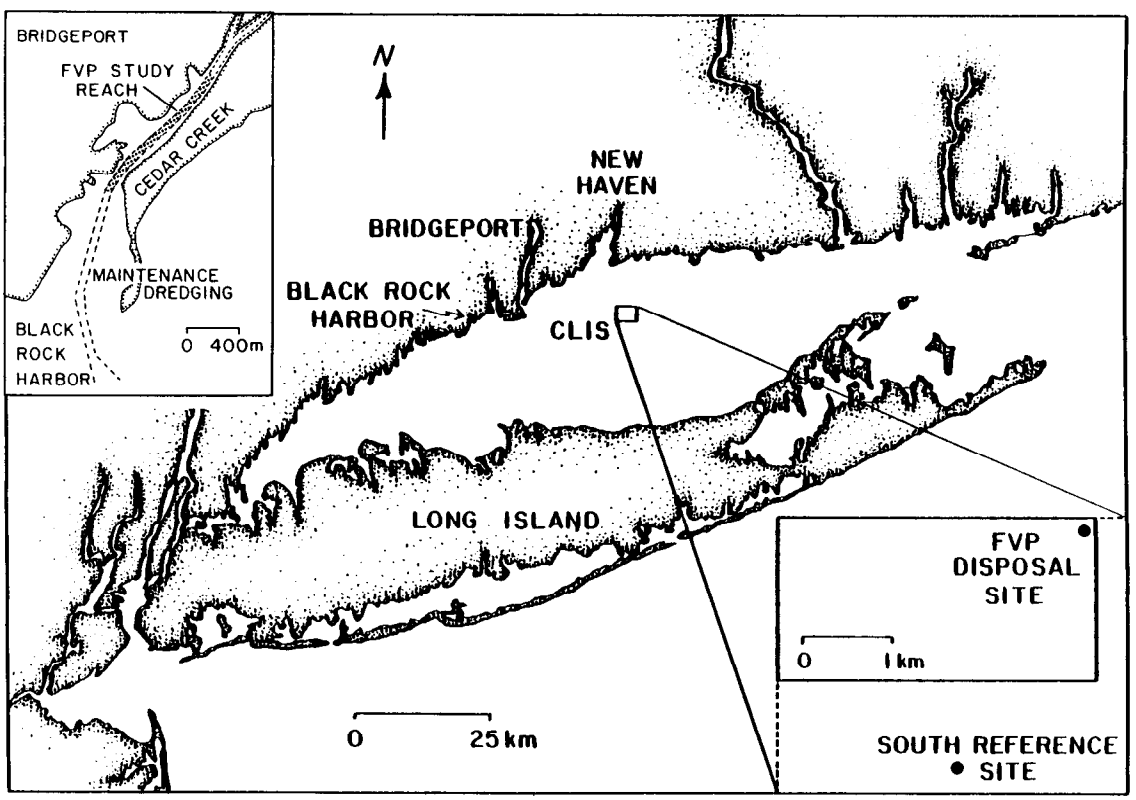

Figure 1. Central Long Island Sound disposal site and Black Rock Harbor dredge site

ongoing disposal activities occurring during the study period. This was necessary to ensure a point source of contamination. The uniformity of physical, chemical, and biological properties of the disposal site prior to disposal allowed detection of changes in these properties due to the disposal of the dredged material. Finally, the stations used to study the biological effects in this study were selected along the primary axis of current flow to represent a gradient of potential exposure for the biota (Figure 2).

9. The spatial scale of this study was near-field and limited to the immediate vicinity of the disposal site. A primary assumption was that the mound of dredged material constituted a point source of contamination. The temporal scale for the study was 4 years, which included a year of predisposal data collection to define seasonal patterns in the physical, chemical, and biological variables and 3 years of postdisposal data collection to address the objectives of the program and to evaluate the long-term impacts of the disposal operation on the surrounding benthic communities.

10. The dredging site was Black Rock Harbor (BRH), located in Bridgeport, Conn., where maintenance dredging provided a channel 46 m wide and

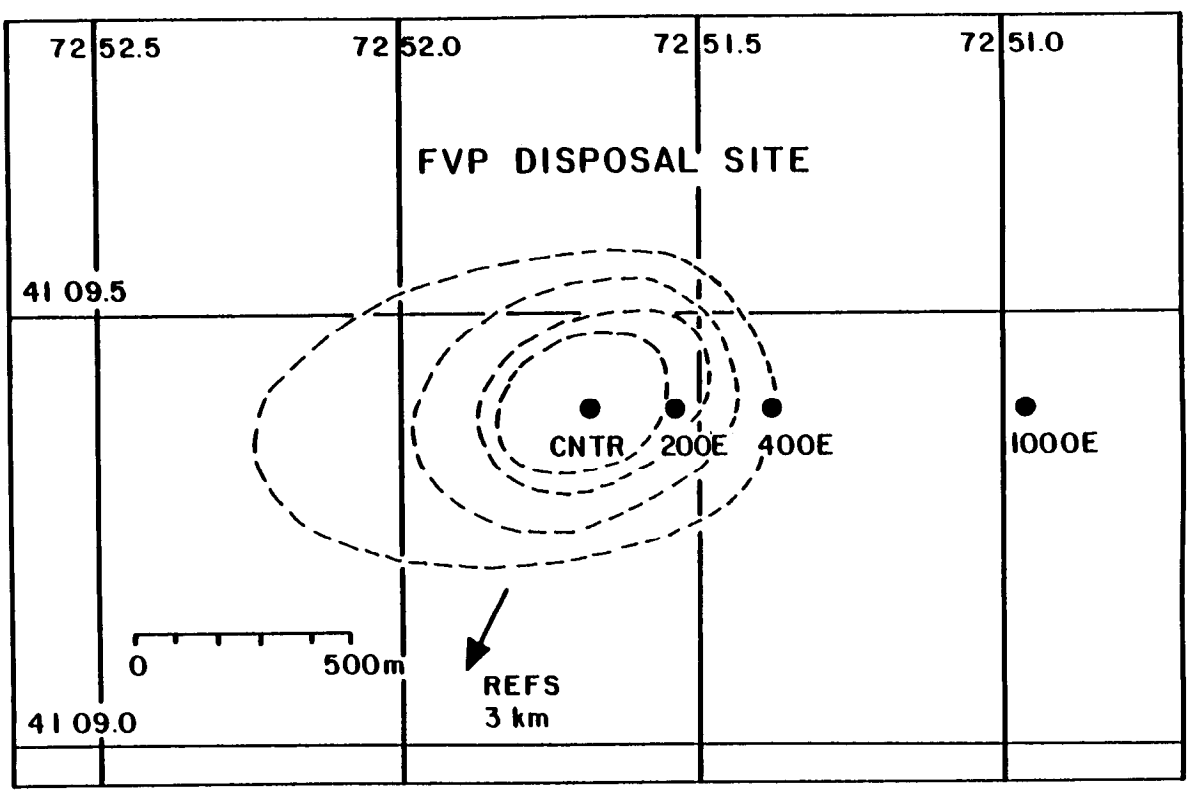

Figure 2. FVP disposal site and station locations

5.2 m deep at mean low water (Figure 1). Approximately 55,000 m^3 of material was dredged during April and May 1983 and disposed in 20 m of water in the northeastern corner of the CLIS disposal site.

11. The dredged material from BRH contained substantial concentrations of both organic and inorganic contaminants (Rogerson, Schimmel, and Hoffman 1985). Polychlorinated biphenyls (PCBs) were present in the dredged material at a concentration of 6,400 ng/g, and polynuclear aromatic hydrocarbons (PAHs) with molecular weights between 166 and 302 were present at concentrations ranging from 1,000 to 12,000 ng/g, respectively. Alkyl homologs of the PAHs were also present in the dredged material at concentrations between 1,000 and 13,000 ng/g. Inorganic contaminants of toxicological importance present in the dredged material include copper (2,900 µg/g), chromium (1,480 µg/g), zinc (1,200 µg/g), lead (380 µg/g), nickel (140 µg/g), cadmium (24 µg/g), and mercury (1.7 µg/g).

Project Scope

12. The FVP is unique among marine research studies for several reasons. The program objectives were directly focused on addressing specific limitations in the methodologies and interpretive framework of the current regulatory process. Among the program strengths were the following: (a) a suite of biological endpoints using the same material was developed and evaluated; (b) the biological tests represented different levels of biological organization; (c) the tests were conducted under both laboratory and field exposure conditions; (d) the tissue residues were examined concurrently with measurements of biological effects; (e) the duration of the study was adequate to evaluate the use of community responses as a benchmark against which other biological responses could be compared; and (f) the project was a site- and waste-specific case study for the application and evaluation of the components of a risk assessment, including the development of methodologies for predicting and measuring field exposures in the water column and benthic compartments. Limitations of this study were: (a) only one dredged material was evaluated, which constrained certain types of comparisons; (b) the size of the study put limits on the extent to which any given objective was examined; and (c) the resources allocated to determine field exposures were limited. The latter is particularly important because the laboratory-field comparisons and

the risk assessment process both require accurate predictions of environmental exposures.

Laboratory-to-Field Comparisons

13. The field verification of laboratory test methods was designed to compare the exposure-response relationships measured in both the laboratory and the field. Exposure for the purposes of this discussion includes the total dredged material with all of its contaminants. Specific contaminants are used as "tracers" to verify the exposure environment, which is described in terms of BRH dredged material, and to illustrate exposure-response relationships between the laboratory and the field. The specific contaminants are a subset of a comprehensive suite of chemicals analyzed in this study and were selected based upon their environmental chemistry and statistical representativeness. The use of specific contaminants in no way implies a cause-and-effect relationship between contaminant and response.

14. Exposure in open marine systems is characterized by highly dynamic temporal and spatial conditions and cannot be completely replicated in laboratory systems. Consequently, the approach chosen for this program was to develop laboratory exposure-response data using only general field exposure information.

Residue-Effects Comparisons

15. Determining the relationship between contaminant tissue residues resulting from bioaccumulation and the biological responses measured is a principal objective of this program. Such relationships do not in any way imply cause and effect, but rather seek to determine the statistical relationship between an effect and any associated residues. The approach used is to determine specific contaminant residues in the tissues of the organisms as a result of exposure to the whole dredged material in both the laboratory and the field. These residues are determined at the same time that biological responses are being measured. Residue-effect relationships will be described and interpreted for both laboratory and field exposures.

Sister Chromatid Exchange

16. In 1977, the USEPA published final regulations and criteria for ocean dumping (USEPA 1977). Section 227.6 listed constituents prohibited as other than trace contaminants. For the first time, this category included "known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion."

17. At present, however, no genotoxic tests are included in permit programs designed to manage waste disposal in estuarine, coastal, and oceanic environments. Short-term tests are needed to detect mutagenic activity in complex mixtures. Long-term tests are needed to investigate possible effects of somatic and germinal mutations on populations of marine species.

18. Several approaches are possible to detect and study genetic toxicants. Chemical analyses of environmental samples can be performed, but these analyses provide no information on the bioavailability of sediment-sorbed compounds. Furthermore, since many classes of compounds are genetically active, the number of analyses would be extensive. A more direct approach is to look for genetic damage in exposed biota. Because cytogenetic techniques are sensitive and relatively simple, it has been recommended that genetic damage in marine organisms be determined by observing their chromosomes directly (International Atomic Energy Agency (IAEC) 1979; Kligerman 1980).

19. Polychaetes were chosen to work with for several reasons. First, they have chromosomes large enough to observe by light microscopy (Christensen 1980; Pesch and Pesch 1980; Pesch et al. 1985). Second, they are easily handled and provide a continuous supply of experimental material. Third, polychaetes are benthic and of particular interest because sediments are often reservoirs for pollutants. Fourth, they are important food web species for commercially important fishes and may, therefore, contribute to biomagnification of toxicants. Fifth, since they are relatively sedentary, field-collected specimens would be representative of the area being sampled. Finally, some species of polychaetes are recognized as pollution indicators, particularly pollution associated with organic loading (Reish 1960, 1972; Wass 1967).

20. The cytogenetic technique of choice is sister chromatid exchange (SCE). An SCE represents the breakage and reciprocal exchange of identical DNA material between the two sister chromatids of a chromosome.

This was originally demonstrated using tritium-labeled DNA (Taylor, Woods, and Hughes 1957). Differential staining methods for light microscopy were developed approximately 10 years ago (Latt 1974; Perry and Wolff 1974). These new techniques transformed SCE from a limited, research tool into one which could be applied extensively to the study of environmental mutagenesis.

21. The usefulness of SCE as an indicator of DNA damage is based on both empirical and biological evidence. Much SCE data exist showing dose responses to known mutagens in both in vitro and in vivo test systems (Latt et al. 1981). The response itself indicates a direct effect on DNA material; SCE is a visual consequence of mutagens that affect a four-strand exchange in DNA. This has been demonstrated in the plant *Vicia faba* (Kihlman and Kronberg 1975), in Chinese hamster cells (Wolff and Perry 1975), and in human cells (Wolff et al. 1975). The formation of an SCE involves the breakage of a chromosome and the subsequent recombination of the four DNA strands. SCE responses have been correlated with induced point mutations and may be useful as a quantitative indicator of mutagenesis (Carrano et al. 1978).

22. Several studies have shown that SCE is a more sensitive method for detecting mutagens and carcinogens than traditional chromosome and chromatid observations (Latt 1974; Perry and Evans 1975; Solomon and Bobrow 1975; Bloom 1978). The SCE response has been recommended for environmental application by the USEPA's Gene-Tox Program (Latt et al. 1981). The application of SCE to polychaetes has created a new tool that is both relevant and practical for the study of genetic problems in marine environments (Pesch, Pesch, and Malcolm 1981). With the in vivo SCE assay, complex wastes can be tested under conditions which better represent actual environmental situations.

23. This report evaluates the use of SCE to measure mutagenic activity associated with contaminated dredged material. The SCE technique was applied to *Nephtys incisa*, an infaunal polychaete dominant in the benthic community of Long Island Sound. The SCE response was measured both in *N. incisa* exposed in the laboratory to suspended and bedded phases of sediments dredged in Black Rock Harbor, Conn., and in *N. incisa* sampled along a transect of stations at the CLIS site where BRH material was disposed.

PART II: MATERIALS AND METHODS

Laboratory Methods

Sediment collection

24. Two sediment types were used to conduct laboratory tests for the field verification studies. The reference (REF) sediment was collected from the South Reference site in Long Island Sound (40°7.95' N and 72°52.7' W) by Smith-MacIntyre grab (0.1 m^2), press sieved through a 2-mm sieve, and stored at 4° C until used. Prior to dredging, contaminated sediment was collected from Black Rock Harbor (41°9' N and 73°13' W) with a gravity box corer (0.1 m^2) to a depth of 1.2 m, thoroughly mixed, press sieved through a 2-mm sieve, and refrigerated (4° C) in barrels until used. Details of sediment collection and storage procedures may be found in Rogerson, Schimmel, and Hoffman (1985).

Organism collection and holding

25. *Nephtys incisa* for laboratory studies were collected with a Smith-MacIntyre grab sampler (0.1 m^2) at the South Reference site (Figure 1). The sediment containing the *N. incisa* was brought to the laboratory where it was sieved, and the *N. incisa* were picked and sorted by size. Tests were conducted with individuals 0.5 to 1.5 cm in length for SCE and 2.0 to 4.0 cm for bioaccumulation. These individuals were placed in REF sediment, in flowing seawater, and were acclimated at a rate of 1° C per day to 20° C. They were fed powdered prawn flakes, ad libitum, during this period.

Dosing systems, exposure chambers, and experimental designs

26. Two different dosing systems, exposure chambers, and experimental designs are described in this section. One was used for the SCE experiments; the other was used for a bioaccumulation experiment. It was intended originally to observe these responses concurrently in the same experiment. However, during the bioaccumulation experiment, a seawater quality problem in the form of a massive bloom of a tiny phytoplankton alga (Hargraves 1986) interfered with the SCE observations. The SCE observations are dependent on cell division. Cell division rate is a sensitive response to stress in worms (Pesch, Pesch, and Malcolm 1981). If the worms are stressed significantly, there will be few, if any, labeled chromosomes to observe. The worms were

stressed by the aberrant phytoplankton bloom during the bioaccumulation experiment; therefore, the SCE observations were not successful. The bioaccumulation experiment is included herein because the data assist in the understanding of the *N. incisa* reaction to BRH suspended sediments and provide a link to field exposures.

27. Suspended sediment dosing system for SCE. Laboratory studies required the construction of two identical sediment dosing systems to provide simultaneously either BRH or REF material as suspended sediment. Each dosing system (Figure 3) consisted of a conical-shaped slurry reservoir placed in a chilled fiberglass chamber, a diaphragm pump, a 4-ℓ separatory funnel, and several return loops that directed the particulate slurry through dosing valves. The slurry reservoirs (40 cm in diameter by 55 cm high) contained 38 ℓ of slurry comprising 36 ℓ of filtered seawater and 2 ℓ of either BRH or REF sediment. The fiberglass chamber (94 cm by 61 cm by 79 cm high) was maintained between 4° and 10° C using an externally chilled water source to minimize microbial degradation during the test. Polypropylene pipes (3.8 cm in diameter) extended to the bottom of the reservoir cones and were connected to pumps (16- to 40-ℓ/min capacity) fitted with Teflon diaphragms. These pumps

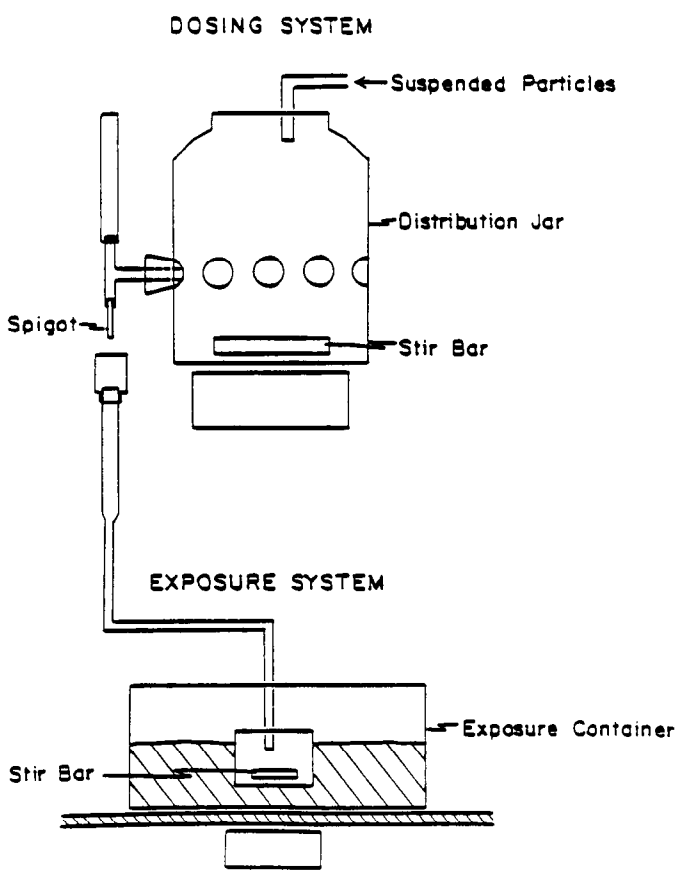

Figure 3. Suspended sediment dosing system

were used to circulate the slurry while minimizing abrasion that might produce changes in the physical properties (e.g., particle size) of the material. The entire dosing system was maintained under argon gas to minimize oxidation of the sediments.

28. The slurry was pumped up to separatory funnels and returned via an overflow to the reservoir through polypropylene pipes. The separatory funnel provided the constant head pressure needed to circulate the slurry through Teflon tubing to the dosing valves where the slurry was mixed with seawater to provide the desired concentrations for the toxicity tests (Figure 4). Narragansett Bay seawater filtered (to 15 μ) through sand filters was used. Actual concentrations of suspended sediments in the test chambers were determined periodically by dry weights (Lake, Hoffman, and Schimmel 1985).

29. Exposure chamber for SCE. The tests were conducted in glass crystallizing dishes (150 by 75 mm). Each dish contained a smaller glass

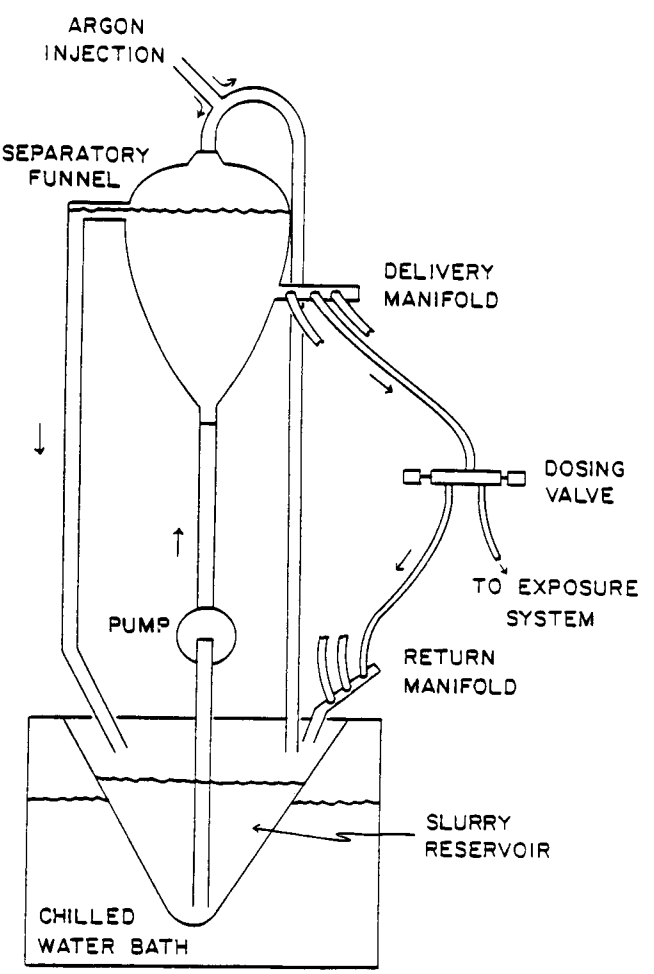

Figure 4. Distribution and exposure system used for laboratory SCE experiments

crystallizing dish (60 by 35 mm) in the center of the larger dish. A Teflon-coated stir bar was placed in the small dish in the center, which received the inflow water, to keep the particulate material in suspension (Figure 4). The inflow water flowed out of the central dish over the sediment surface, and overflowed the edge of the crystallizing dish. Each dish contained 400 mℓ of sediment (2.5 to 3.5 cm deep).

30. Experimental design for SCE. This study was set up as a randomized complete block design with three replicates (blocks). Each replicate contained four exposure regimes. Due to limitations of space and availability of animals, the three replicates were run at successive times. Exposure conditions for the bedded phase portion of the suspended particulate tests were either 100-percent REF or 100-percent BRH. These two bedded phase exposure conditions in combination with the two suspended sediment exposures, REF or BRH at about 200 mg/ℓ (dry weight), gave a total of four treatments. The treatment with REF sediment in both bedded and suspended phases served as the control. Each treatment had 15 worms. The worms were fed prawn flakes (ADT-Prime, Aquatic Diet Technology, Brooklyn, N. Y.) in a suspension of seawater, which was pumped by peristaltic pump into the distribution chamber of the dosing system. The amount fed was 130 mg per test chamber per day. This amount of food was determined in prior feeding studies with *N. incisa*.

31. During the tests, all dishes were examined daily for the appearance of any worms on the surface of the sediment. Stressed worms will come to the surface of the sediment and remain there. On the last day of the test, observations were made on the burrows visible through the sides of the dishes and the depth of suspended material deposited on top of the solid phase was measured. Then the sediment was sieved (0.335-mm mesh) and the worms retrieved and counted.

32. All tests were conducted with sand-filtered Narragansett Bay seawater at 20° C and approximately 30 g/kg salinity. Seawater flow rates were about 35 mℓ/min. The photoperiod was a 14:10-hr light-dark cycle.

33. Suspended sediment dosing system for bioaccumulation. The suspended sediment dosing system used for the bioaccumulation experiment was the same system used for the SCE experiments. However, the argon gas was omitted. The REF and BRH sediments used in this experiment were oxidized before they were placed into the dosing system. In order to obtain consistent states of oxidation for both REF and BRH sediments, 2 ℓ of sediment were transferred to

an inverted polycarbonate carboy and diluted to 19 ℓ with filtered natural seawater at room temperature and aerated for 3 to 4 days. The contents were transferred to the composite dosing system reservoir and diluted to 38 ℓ with natural seawater. Chemical oxygen demand measurements indicated that this time period was sufficient to satisfy the immediate oxygen demand of the sediments.

34. Exposure chamber for bioaccumulation. In the laboratory tests with *N. incisa*, the dosing system was set to maintain nominal concentrations of 200 mg/ℓ (dry weight) of suspended sediments with seawater flow rates producing five volume replacements per exposure chamber per day. These flow rates meet the minimum recommended by the American Society for Testing and Materials (1980) and were intended to maximize residence time of the suspended sediments in the exposure chambers.

35. A suspended sediment proportional diluter (Figure 5) was used to

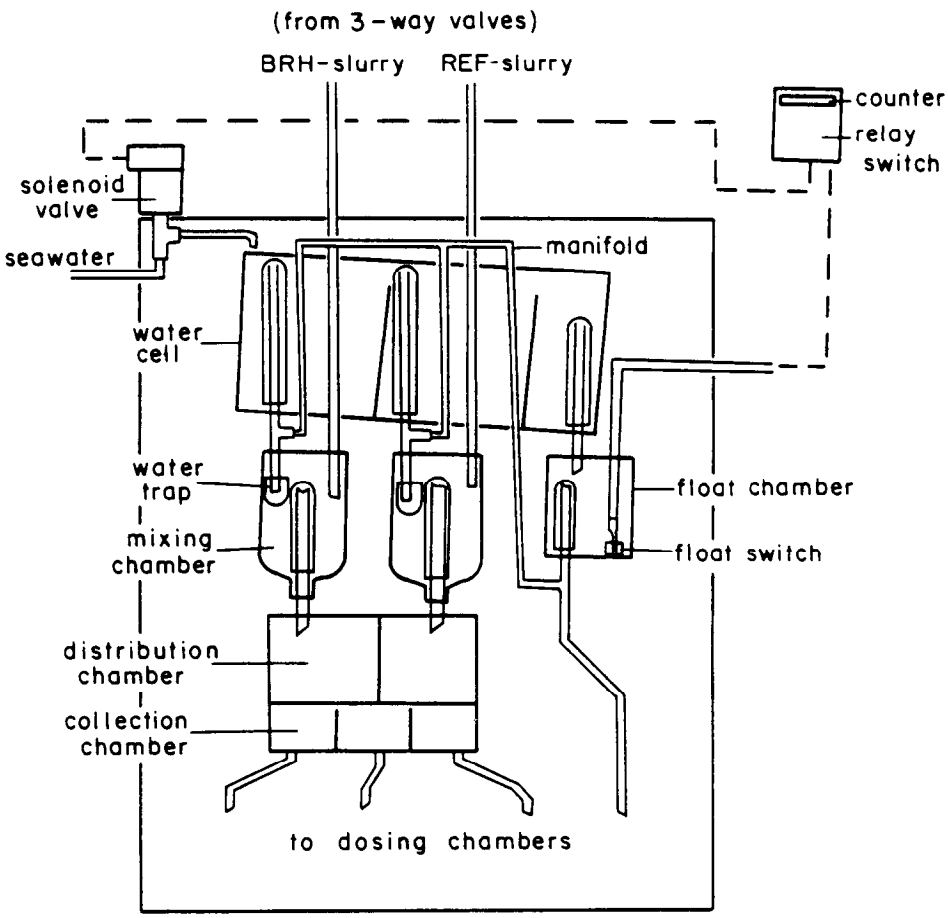

Figure 5. Proportional diluter used to deliver suspended sediment to the *N. incisa* exposure chambers for bioaccumulation study

mix small quantities of concentrated sediment slurries (10 to 20 g/ℓ) from the sediment dosing system with filtered seawater to produce diluted sediment suspensions in the milligrams-per-litre range. It then combined slurries of different types (e.g., REF and BRH sediment suspensions) proportionally to maintain the same concentration of suspended sediment with different ratios of the two sediments.

36. The exposure chamber for *N. incisa* is illustrated in Figure 6.

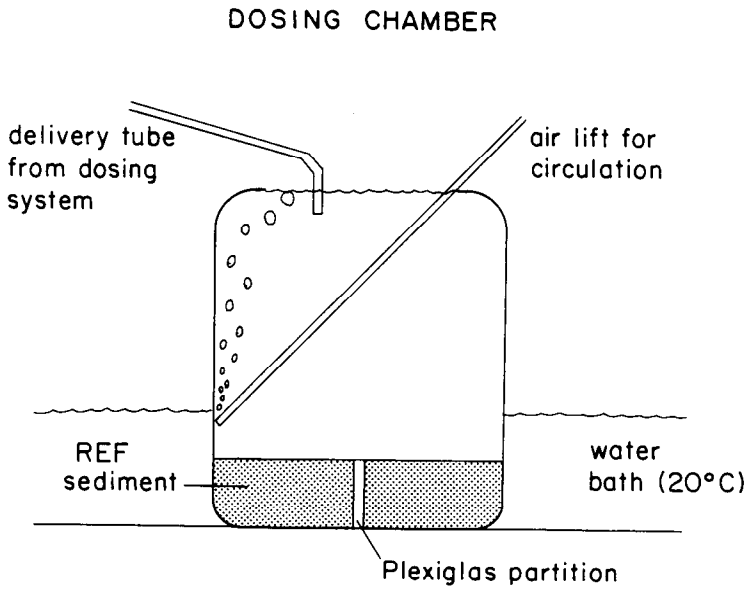

Figure 6. Exposure chamber for bioaccumulation study

Polycarbonate bottles (19 ℓ) used commercially for shipping spring water were cut off at the top. REF sediment (2 ℓ/chamber) was added to a depth of 4 cm, and Plexiglas strips were inserted into the sediment, dividing it into pie-shaped sections. This permitted subsampling without disturbing the entire chamber. Each chamber was filled with filtered seawater at 20° C. After the sediment in the chambers was permitted to settle and equilibrate for about 4 hr, *N. incisa* were added, and an additional 2 hr was allowed for the worms to burrow into the sediment. The delivery tubes from the proportional diluter were then put in place, and a low pressure airlift was turned on to keep the dosed sediments in suspension. This system allowed very little sediment deposition during the course of the experiments. Excess seawater was permitted to overflow the brim of each chamber. Earlier experiments indicated that

once the worms burrowed into clean reference sediment they would not attempt to escape. Therefore, the chamber design used here was considered acceptable.

37. Experimental design for bioaccumulation. This experiment had exposure conditions of 100-, 50-, and 0-percent BRH suspended sediment. Worms were removed at time zero, day 28, and day 42. This experiment was supported with chemical analyses of the seawater and of the *N. incisa*. *Nephtys incisa* were collected on a sieve after removal of a pie-shaped aliquot of bedded sediment from each chamber. Clean reference sediment, without *N. incisa*, was returned to the vacated section to maintain the integrity of the exposure chamber.

38. Suspended sediment, temperature, and salinity were measured routinely during each experiment. Dissolved oxygen (DO) concentrations were not expected to be a problem because of the large volume of the chamber and the use of an airlift. However, DO levels were determined once during each experiment and were never different from saturation. The worms were fed 100 mg of powdered prawn flakes per chamber per day for the duration of each experiment.

Chromosome labeling, laboratory and field

39. To facilitate SCE observations, chromosomes must be differentially stained. Differential staining is a consequence of labeling chromosomes with the base analog 5-bromodeoxyuridine (Brdu) for two cell cycles (Latt 1982). Because the labeling phase must include two cell cycles, the time needed varies according to growth rate. Therefore, this time may differ with species and with age of the test organism. In this study, these times were determined empirically.

40. The labeling phase was done subsequent to the laboratory exposure or directly upon return from field sampling. The only difference between the labeling of laboratory worms and field worms was the temperature of the seawater. Laboratory worms were labeled at 20° C, while the field worms were labeled at ambient seawater temperature, which ranged from 0 to 20° C depending on season. In both cases the worms were exposed to 3 mg/ℓ of Brdu.

41. To maintain healthy worms, *N. incisa* must be held in sediment; therefore, they were placed in clean, fine-grained sand with 3 ℓ of filtered, labeled seawater (30 g/kg). They were held in subdued light. Each treatment had approximately 15 worms, and these were fed 30 mg of prawn flakes every

other day. Each time food was added, 2.3 g of REF sediment was added also, because in prior feeding studies, the presence of suspended sediment was found to enhance growth. The sediment was maintained in suspension by gentle aeration of the seawater. The labeled seawater was renewed every other day. The worms were labeled for 10 to 20 days. Colchicine (0.05 percent) was added to the seawater for the last 15 hr of the labeling period in order to arrest cell division at metaphase.

Slide preparation and staining

42. The following procedure for slide preparation was adapted from Kligerman and Bloom (1977). The worms were removed from the labeling treatments and placed in 100 mℓ of 0.075 M solution of potassium chloride for 1 hr. They were then fixed in three changes of cold ethanol-acetic acid (3:1) for 0.5 hr each. Fixed worms were placed individually in a clean well-slide, and 1 mℓ of 60-percent acetic acid was added. The worm was macerated for approximately 1 min or until the tissue appeared translucent. The material was then drawn up into Pasteur pipettes and applied to clean, hot (45° C) slides. Excess acetic acid was immediately removed from the slides. This procedure produced a monolayer of separated cells on each slide.

43. Slides were stained according to a procedure recommended by Bloom.* Slides with Brdu-labeled chromosomes were stained with 225 μg/mℓ of 32258 Hoechst stain for 10 min (several drops placed on slide and coverslip added), rinsed an excess of McIlvaine's buffer (0.1 M citric acid, 0.2 M disodium phosphate) at pH 8.0, and placed between two black lights for 60 min. The slide was then rinsed in distilled water, air dried briefly, and stained with 2-percent Giemsa in deionized water for 7 min. The slide was rinsed in distilled water, air dried, soaked in xylene, and mounted in Coverbond. Observations were made with a compound microscope at 1250×, oil immersion.

Data collection and analysis

44. The SCE observations were made on 25 second-division (metaphase stage) cells for each treatment unless otherwise noted. Cells were selected under low power, then counted under high power. For each treatment, the individual worms were screened sequentially. Counting continued until a total of 25 cells were counted regardless of the number of individual worms. This

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* Personal Communication, S. E. Bloom, 1981, Cornell University, Ithaca New York.

assumes that organism-to-organism variance was small compared with within-organism variance. This assumption has been tested for these data and found to be true.

45. The data were examined to see whether criteria were met for parametric statistical analysis or whether data transformation was necessary. All of the SCE data in this report were transformed to log 10 (SCE/chromosome + 0.1) prior to statistical analysis. The 0.1 is added because log (0) is undefined. The laboratory data were analyzed by two-way analysis of variance for a randomized complete block design (Snedecor and Cochran 1980).

Field Methods

Organism collection and holding

46. *Nephtys incisa* for field studies were collected at stations REFS, 1000E, 400E, 200E, and CNTR. Station locations were marked with buoys for the duration of this project. While the boat was anchored, a Smith-MacIntyre grab sampler (0.1 m^2) was used to collect bottom sediments. These sediments were wet sieved on deck (nested sieves of 2- and 0.5-mm mesh size), and organisms were collected. Specimens for SCE were placed in sediment and returned to the laboratory for processing.

Exposure

47. Field exposures via tissue residues. The purpose of exposure assessment is to determine the temporal and spatial range of exposure concentrations experienced by populations of interest. The exposure conditions present in the field for *N. incisa* were not as well characterized as they were in the laboratory studies. As a result, the description of *N. incisa* exposure to BRH material in the field is more qualitative than quantitative and is presented in three parts. First, a prediction of field exposure is based on worm tissue residues. The relationship between exposure to BRH sediments and tissue residues was determined in a laboratory experiment. Tissue residues of PCBs as A1254 from worms exposed to 0-, 50-, and 100-percent BRH treatments (200 mg/ℓ suspended sediment) for 42 days in the laboratory were plotted against BRH exposure concentrations. This plot was used to estimate field exposure conditions based on tissue residues of PCBs in field-collected worms. Inherent in this approach is the assumption that organisms have comparable patterns of bioaccumulation in the laboratory and field.

48. Field exposures from physical data. A second analysis calculates the maximum total suspended solids concentrations from 1 m above the bottom to the sediment-water interface. This analysis assumes that the total suspended solids are composed of BRH sediments, and it represents a worst case or upper bound prediction. A third approach calculates the probable amount of BRH sediment exposure at the sediment-water interface based upon the actual contaminant concentrations for each sampling station and date. This analysis assumes that resuspension of surficial sediment is the primary source of total suspended solids at the sediment-water interface.

49. The equation* used to calculate total suspended solids concentrations from the sediment-water interface up to 1 m above the bottom is described as follows:

$$C_z = \left[C_m\ 1 + (C_o - 1)\ e^{-kz}\right] \quad (1)$$

where

C_z = total suspended solids concentration at distance z

C_m = total suspended solids concentration at 1 m above the bottom

C_o = enrichment factor (C_z/C_m when z = 0)

-k = rate of change in total suspended solids concentration as a function of z

z = distance from the bottom, m

Given the total suspended solids concentration at 1 m above the bottom, the equation predicts an exponential increase in suspended solids concentration at distances from 1 m above the bottom to the sediment-water interface. *Nephtys incisa* feeds at the sediment-water interface.

50. The total suspended solids concentrations for these analyses were selected to represent average and storm conditions empirically determined from an in situ continuous monitoring platform deployed 1 m above the bottom at the disposal site (Bohlen and Winnick 1986; Munns et al. 1986). Enrichment factors were likewise empirically determined from acoustic profilometer data collected between the sediment-water interface and 1 m above the bottom (Bohlen and Winnick 1986; Munns et al. 1986).

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* Personal Communication, John F. Paul, 1986, ERLN.

Maximum upper bound estimate

51. For the purposes of the maximum upper bound analyses, it was assumed that the populations at risk are located off the mound and aligned with the mean direction of current flow. The route of contaminant exposure is assumed to be through the transport of resuspended BRH sediments. These total suspended solids are composed of resuspended Long Island Sound sediments, as well as BRH sediment resuspended from the disposal site. Since the intent of these analyses is to create a maximum upper bound set of exposure conditions, it was assumed that the suspended solids concentration was composed, in total (100 percent), of resuspended BRH sediment.

Probable exposure estimate

52. It was not within the scope of this program to provide a continuous temporal record of the percent contribution of BRH sediments to the total suspended solids load. Consequently, a second set of analyses was designed to estimate the percentage of BRH sediment that could have comprised the total suspended solids concentration at the sediment-water interface for each station and how these concentrations changed with time throughout the study. The proportions of BRH dredged material in the surficial sediments at each station and date were estimated by comparing the concentrations of selected contaminants measured in the 0- to 2-cm layer of sediment cores collected, post-disposal, at the FVP site (Appendix A, Tables A1-A12). These field concentrations were compared with the barrel concentrations to determine a percentage as follows:

$$\text{Percentage BRH Sediment} = (C - REF/BRH - REF) \times 100 \qquad (2)$$

where

C = concentration of contaminant in the sediment core

REF = concentration of contaminant in REF sediment

BRH = concentration of contaminant in BRH sediment (barrel)

The percentage BRH sediment values were calculated for each station and date using the 11 different contaminants, the details of which are shown in Appendix A, Table A13. To achieve a BRH-suspended sediment concentration that reflects the surficial sediment contaminant levels for each station and date, the total suspended solids concentrations predicted for the sediment-water interface were multiplied by the estimated proportions of BRH sediment.

Chemical Methods

Analytical methods

53. The analytical methods used in this study are presented here in summary form. More detailed descriptions of the analytical methods are available in Lake, Hoffman, and Schimmel (1985). Most of these methods represent extensive modifications of USEPA standard methods developed for freshwater and wastewater samples. It was necessary to modify these methods to analyze the types of matrices in this study. These methods were inter-calibrated to ensure the quality of the data.

Organic sample preparation

54. Samples of sediment, suspended particulates, and organisms were extracted by multiple additions of increasingly less polar organic solvents using a tissue homogenizer. These mixtures were separated by centrifugation between additions; polar solvents were removed by partitioning against water; and the extracts were desulfured with activated copper powder when required. The extracts were then passed through a precolumn containing activated silica gel. Samples of both filtered and unfiltered seawater were solvent extracted in separatory funnels, and the extracts were saved. Foam plugs containing the dissolved organic contaminants from water samples were extracted with organic solvents. All of the above extracts were subjected to column chromatography on deactivated silica gel to separate analytical fractions and were volume reduced carefully prior to analysis.

Organic analysis

55. Electron capture gas chromatographic analyses for PCBs were conducted on a Hewlett-Packard 5840 gas chromatograph equipped with a 30-m DB-5 fused silica column. Samples were quantified against an A1254 standard because the distribution of PCB congeners in the dredged material closely matched that distribution, as did the distribution in organisms at steady-state.

56. Gas chromatograph/mass spectrometric analyses were conducted with a Finnigan Model 4500, also equipped with a 30-m DB-5 fused silica capillary column. The mass spectrometer was operated through a standard Incos data system and was tuned at all times to meet USEPA quality assurance specifications.

57. All instruments were calibrated daily with the appropriate standards. The concentrations of the standards used were chosen to approximate

those of the contaminants of interest, and periodic linearity checks were made to ensure the proper performance of each system. When standards were not available, response factors were calculated using mean responses of comparable standards. Blanks were carried through the procedure with each set of samples, and a reference tissue homogenate was analyzed with every 12 to 15 tissue samples.

Organic data reduction

58. As stated above, PCBs were quantified as A1254 because the sample patterns closely resembled that profile. This allowed a convenient way of reporting these data without treating the voluminous data that would have resulted from measuring some 55 congener peaks by electron capture detector. Likewise, a method was sought to summarize the PAH data. The 35 individual PAH parent and alkyl homolog compounds and groups of compounds measured in this study are listed in Appendix B. Each PAH of the same molecular weight, both parents and alkyl homologs, can be summed to yield 9 PAH parent sums and 5 alkyl homolog sums. Although useful, this only reduced the data to 14 PAH variables, which was not sufficient. Since the distribution of PAHs differed greatly in both quantity and quality between Long Island Sound sediments and the BRH dredged material, statistics were sought that would retain significant quantitative and qualitative information. The quantitative statistic chosen was the simple SUM of all measured PAHs, and a qualitative descriptor was chosen by analogy with the center of mass concept from elementary physics and called a centroid (CENT). In this case, CENT describes the "center of mass" of the PAH distribution, and is in units of molecular weight. It is the concentration-weighted average molecular weight of any particular PAH distribution. This statistic can be used to readily distinguish two different sources of PAH distributions, one with predominately heavy molecular weight pyrogenic compounds, and one with more lighter molecular weight petrogenic compounds. These distributions are typically found in Long Island Sound at REFS and BRH, respectively. A major value of this statistic is that it enables one to readily distinguish these two sources when their concentrations are nearly equal. The formulas for calculating these are shown in Appendix B.

Inorganic sample preparation

59. Sediment was prepared for inorganic analysis by elution at room temperature with 2N HNO_3. The samples were filtered through Whatman #2 filter

paper. Organisms were totally digested in concentrated HNO_3 at 60° C and filtered through Whatman #2 filter paper.

60. Cadmium, nickel, lead, and copper were concentrated and separated from both the unfiltered and filtered seawater fractions by coprecipitation (Boyle and Edmond 1975). The remaining metals (chromium, iron, manganese, and zinc) were analyzed by heated graphite atomization atomic absorption (HGA-AA) via direct injection. Samples of suspended particulates on Nucleopore (0.45 μ) filters were eluted with 2N HNO_3 and analyzed by HGA-AA.

Inorganic analysis

61. All flame atomization atomic absorption (FA-AA) was conducted with a Perkin-Elmer (Model 5000) atomic absorption spectrophotometer. All HGA-AA determinations were conducted with Perkin-Elmer Model 500 or 2100 HGA units coupled to Perkin-Elmer Model 5000 or 603 atomic absorption instruments, respectively. The Model 5000 AA was retrofitted with a Zeeman HGA background correction unit, and the Model 603 was equipped with a D2 arc background correction system.

62. The FA-AA and HGA-AA instrument operating conditions are similar to those described in USEPA (1979) and those in the manufacturers' reference manuals. The AA instruments were calibrated each time samples were analyzed for a given element. Sample extracts were analyzed a minimum of twice to determine signal reproducibility. Quality assurance checks, conducted after every 15 samples, were analyzed by the method of standard addition and by analyzing one procedural blank.

Contaminant selection

63. Chemical analyses performed in this study characterize the organic and inorganic constituents in the dredged material, provide information on the laboratory and field exposure environments, provide insight into the processes governing contaminant movement within and between environmental compartments, and determine which contaminants were accumulated by organisms. Historically, bulk sediment analyses have been used to characterize dredged material. More recently, dredged material must be analyzed for USEPA's priority pollutants to determine if hazardous substances are present and, if so, in what concentrations. While both of these approaches were used in this study, neither addresses the issue of bioavailability and the potential for contaminants to bioaccumulate. In this study, bioavailability was determined by examining the types and distributions of contaminants that bioaccumulated in laboratory

studies (Rogerson, Schimmel, and Hoffman 1985). Based upon the contaminant profile for the dredged material and residue data, the contaminants selected for detailed analyses throughout the study included PCBs, PAHs, the pesticide ethylan, and eight metals.

64. A representative subset of chemicals was selected for discussion throughout the study. The criteria used in selecting this subset included chemical properties, contaminant representativeness and behavior in various compartments, and statistical analyses of the distributions of the complete suite of chemicals analyzed in the program.

65. Multivariate clustering analyses were performed on the chemical data in an attempt to define groups or clusters of chemicals that behaved in a statistically similar manner. No assumptions were made concerning the behavior, interactions, or dynamics of chemicals between compartments; therefore, each compartment was analyzed separately. Five compartments were identified from field and laboratory data for statistical analysis. Of these, the surficial sediments and the unfiltered, particulate, and dissolved water column fractions described exposure conditions experienced by infaunal and pelagic organisms. The remaining compartment consisted of tissue residues in organisms.

66. The data were further partitioned into inorganic and organic analyses. The inorganic analyses generally consisted of 8 variables, whereas the organic analyses contained 61 variables. The clusters of chemicals identified through the statistical analyses agreed well with those contaminants selected, based on chemical properties and environmental behavior. The subset of chemicals selected as representative included six organic compounds, four metals, and two summary statistics.

Statistical Analysis Methods

67. The primary objective of the FVP was to compare laboratory and field responses under similar exposure conditions. Because of the highly dynamic temporal and spatial conditions in the field, the exposure environment can be given only boundaries and cannot be assigned specific values, as is the case for laboratory studies. Consequently, the degree to which laboratory exposure-response relationships concur with those derived from field data can be described only qualitatively. That does not preclude the use of

inferential statistical procedures to explore those laboratory and field relationships for which the appropriate quantitative information is available. However, the nature of this project was such that descriptive and exploratory statistics were often the most appropriate techniques to illustrate relations and trends. Simple graphic representations of variables were all that was necessary to illustrate a relationship. In addition, multivariate techniques, such as cluster analysis, were the most appropriate techniques to elucidate more complex relationships between groups of selected variables.

68. Prior to making comparisons between laboratory and field effects, it was necessary to establish whether field exposure boundaries were similar to those measured in the laboratory. Assuming that tissue residue and exposure are closely related, this was accomplished by examining the tissue residues of all worms from laboratory and field exposures together, independent of exposure concentration or station location and date. An agglomerative hierarchical cluster analysis was performed on the ten selected chemical contaminants and the two summary statistics using the SAS cluster procedure (SAS 1985) to establish which tissue residues among all the laboratory treatments and field stations were most similar. The clustering procedure used was the average linkage method, which uses unweighted pair-groups with arithmetic averages on squared distances between samples. Prior to analysis, residue data were normalized using standard deviations from the mean. This procedure ensured that each variable was weighted equally, even if its absolute value was orders of magnitude different from another variable.

69. The relationship between SCE and tissue residue values in the field samples was explored by regressing and plotting the mean SCE value for each sample against the corresponding mean tissue residue (Snedecor and Cochran 1980). This procedure was completed individually for each of the ten selected chemical contaminants and the two summary statistics.

PART III: RESULTS

Laboratory Results

Exposure

70. Dosing system monitoring. During the *N. incisa* laboratory experiment for SCE response, the exposure system was monitored for total suspended sediments, temperature, and salinity. These data are presented in Table 1. Similar measurements were made on the exposure system during the 42-day *N. incisa* bioaccumulation experiment. These data are presented in Table 2. In general, the exposure systems maintained the suspended sediment concentrations close to the nominal 200 mg/ℓ. Temperature and salinity values were stable at approximately 20° C and 30 g/kg, respectively. DO concentrations were checked once during each experiment and were never different from saturation.

Table 1

Measured Suspended Sediment Concentrations (Dry Weight) and Exposure Conditions for the Laboratory SCE Tests with *N. incisa*

Treatment	Suspended Sediment Concentration, mg/ℓ $\bar{x}$ ± SD	Seawater Temperature Range, °C	Seawater Salinity Range, g/kg
	Replicate 1		
REF/REF	217 ± 86	21.0 - 22.0	30.0 - 31.0
BRH/REF	190 ± 61	21.0 - 22.0	30.0 - 31.0
REF/BRH	217 ± 86	21.0 - 22.0	30.0 - 31.0
BRH/BRH	190 ± 61	21.0 - 22.0	30.0 - 31.0
	Replicate 2		
REF/REF	199 ± 73	19.8 - 22.0	30.0 - 31.8
BRH/REF	226 ± 48	19.8 - 22.0	30.0 - 31.8
BRH/BRH	226 ± 48	19.8 - 22.0	30.0 - 31.8
	Replicate 3		
REF/REF	211 ± 87	20.5 - 22.5	30.0 - 31.0
BRH/REF	171 ± 53	20.5 - 22.5	30.0 - 31.0
REF/BRH	211 ± 87	20.5 - 22.5	30.0 - 31.0
BRH/BRH	171 ± 53	20.5 - 22.5	30.0 - 31.0

Table 2

Measured Suspended Sediment Concentrations (Dry Weight) and Exposure Conditions for Laboratory Bioaccumulation Test with *N. incisa* (Means ± Standard Deviation)

Treatment	Suspended Sediment Concentration, mg/ℓ	Seawater Temperature °C	Seawater Salinity g/kg
100% BRH	210 ± 23	19.8 ± 0.53	30.9 ± 0.70
50% BRH	184 ± 19	19.8 ± 0.53	30.9 ± 0.70
0% BRH	190 ± 21	19.8 ± 0.53	30.9 ± 0.70

71. Chemical monitoring of seawater. During the 42-day bioaccumulation experiment, seawater and *N. incisa* from the exposure chambers were sampled for chemical analysis. Seawater chemical monitoring data are presented in Table 3. The dosing system malfunctioned for 2 days spilling BRH sediments into all treatments. The day 18 chemistry samples were taken during this period. The problem was corrected, and the system performed normally for the remainder of the test. The seawater chemistry data confirm that *N. incisa* received a graded exposure to BRH sediments during most (40 of 42 days) of the experiment.

Chemical analysis of test sediments

72. The contaminant-specific analysis of the BRH and REF sediments is presented in summary form in Table 4 for the representative subset of chemical compounds discussed in this report. These analyses demonstrate clearly the differences in contaminant concentration between the two sediments. These differences facilitated the tracing of these contaminants in the exposed biota.

Tissue residue

73. *Nephtys incisa* tissues from suspended sediment laboratory exposures were analyzed for a suite of organic and inorganic contaminants found in BRH sediment. These tissue residues were measured on samples from days 0, 28, and 42 of the experiment. The summary statistics SUM and CENT of the PAHs were also calculated for each of these sampling dates. The tissue residue data for the representative subset of chemical compounds are presented graphically in Figures 7-12.

Table 3

Chemical Analysis of Seawater in Exposure Chambers of the Bioaccumulation Experiment Exposing *N. incisa* to BRH Sediment

Experiment Day	Treatment	Total PCB (ng/ℓ as A1254)	Total Metals µg/ℓ Cu	Cd	Cr
3	100% BRH	NS*	407	5.4	245
	50% BRH	NS	256	3.2	159
	0% BRH	NS	15	0.1	15
6	100% BRH	1,170	NS	NS	NS
	50% BRH	590	NS	NS	NS
	0% BRH	79	NS	NS	NS
18**	100% BRH	340	307	3.6	181
	50% BRH	510	208	3.5	125
	0% BRH	700	134	2.2	89
32	100% BRH	NS	357	5.0	203
	50% BRH	NS	171	2.6	106
	0% BRH	NS	15	0.1	16
42	100% BRH	1,920	NS	NS	NS
	50% BRH	980	NS	NS	NS
	0% BRH	12	NS	NS	NS

* Not sampled.

** Dosing system malfunctioned for 2 days spilling BRH sediments into all treatments.

74. Although these data are not discussed in detail (see Lake et al. 1987), some general observations are made. The tissue residue concentrations of all the organic compounds increased with increasing exposure. The PAHs, with the exception of fluoranthene, reached their highest measured tissue concentrations at day 28 and exposure concentrations of 100-percent BRH (200 mg BRH/ℓ). The residue concentrations of phenanthrene and benzo(a)pyrene declined by 30 and 50 percent, respectively, by day 42. The tissue residue concentration of PCBs reached an apparent steady-state at the 50-percent BRH (100 mg BRH/ℓ) exposure by day 28 although there was a continued increase at 100-percent BRH (200 mg BRH/ℓ) at day 42. Because of its kinetic, partitioning, and persistence properties, PCB was selected as a "tracer" for BRH material and was used to relate BRH exposure conditions to tissue residues. Not

Table 4

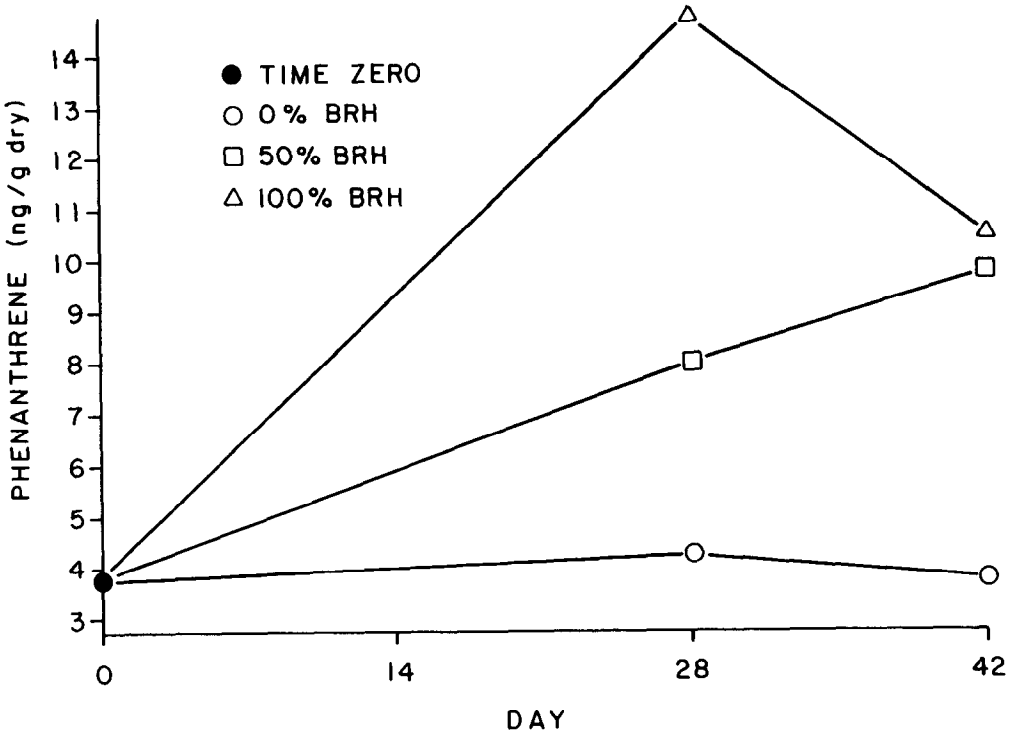
Concentrations of the Ten Selected Contaminants and Two Summary Statistics for Both BRH and REF Sediment (Means ± Standard Deviations)

Chemical Compound	Sediment* BRH	REF
Phenanthrene	5,000 ± 1,800 (15)**	85 ± 17 (12)
Sum of 178 alkyl homologs	28,000 ± 8,300 (15)	170 ± 26 (12)
Fluoranthene	6,300 ± 1,300 (15)	240 ± 33 (12)
Benzo(a)pyrene	3,900 ± 970 (15)	250 ± 28 (12)
Ethylan	4,000 ± 820 (15)	0 ± - (12)
PCB as A1254	6,400 ± 840 (15)	39 ± 4 (12)
SUM of PAHs	142,000 ± 30,000(15)	4,500 ± 520 (12)
CENT of PAHs	232.8 ± 1.7 (15)	249.2 ± 1.7 (12)
Copper	2,900 ± 310 (18)	60 ± 3 (15)
Cadmium	24 ± 0.6 (18)	0.23 ± 0.04 (15)
Chromium	1,480 ± 83 (18)	50 ± 15 (15)
Iron	31,000 ± 2,800 (18)	21,000 ± 1,400 (15)

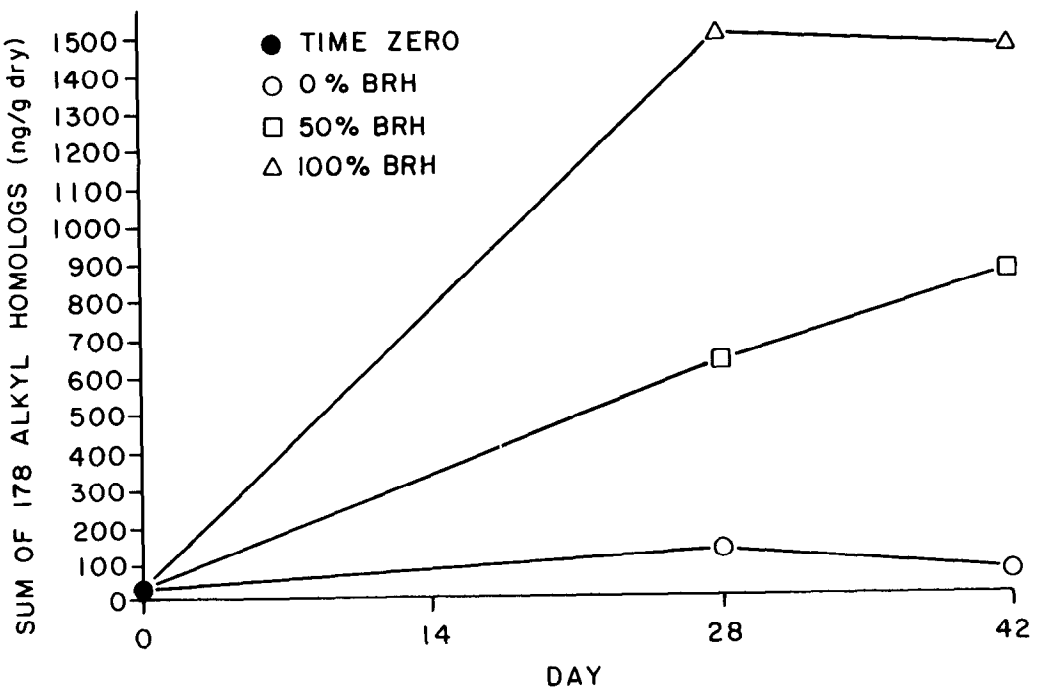
* Units of nannograms gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENT.

** (N) = number of sediment samples analyzed.

all the inorganic compounds produced increased tissue concentrations. Copper and cadmium, which have soluble fractions in seawater, did produce elevated tissue concentrations as a consequence of increased exposure to BRH suspended sediment. Chromium and iron, which are bound to particulates, did not produce elevated tissue concentrations and in fact showed apparent depuration of these compounds from day 28 to day 42 of the experiment.

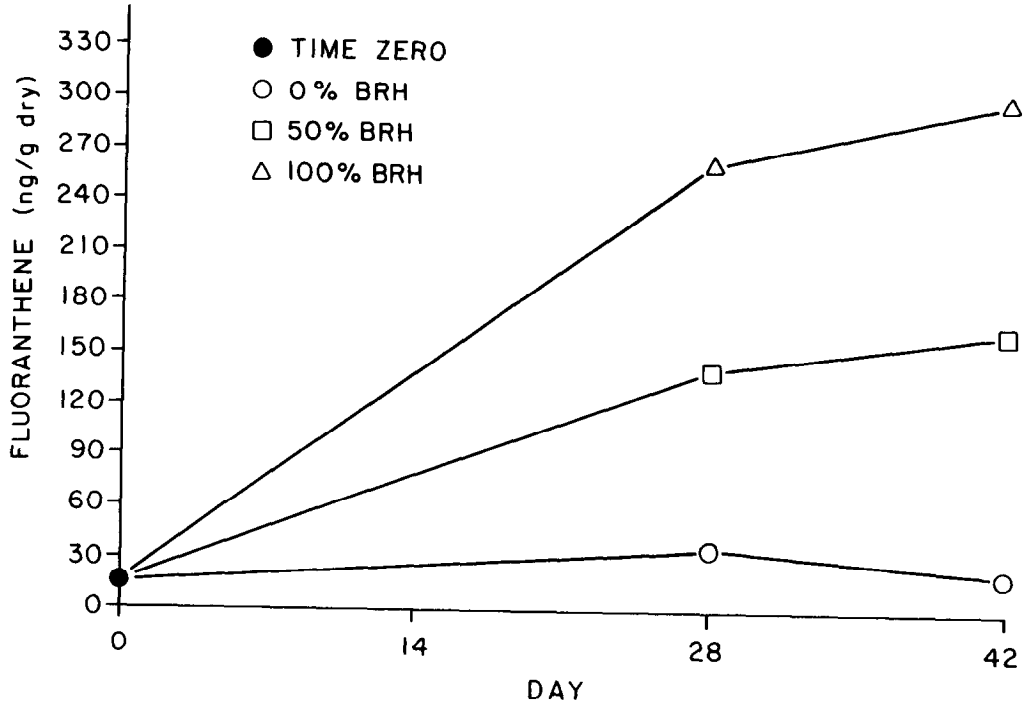


a. Phenanthrene

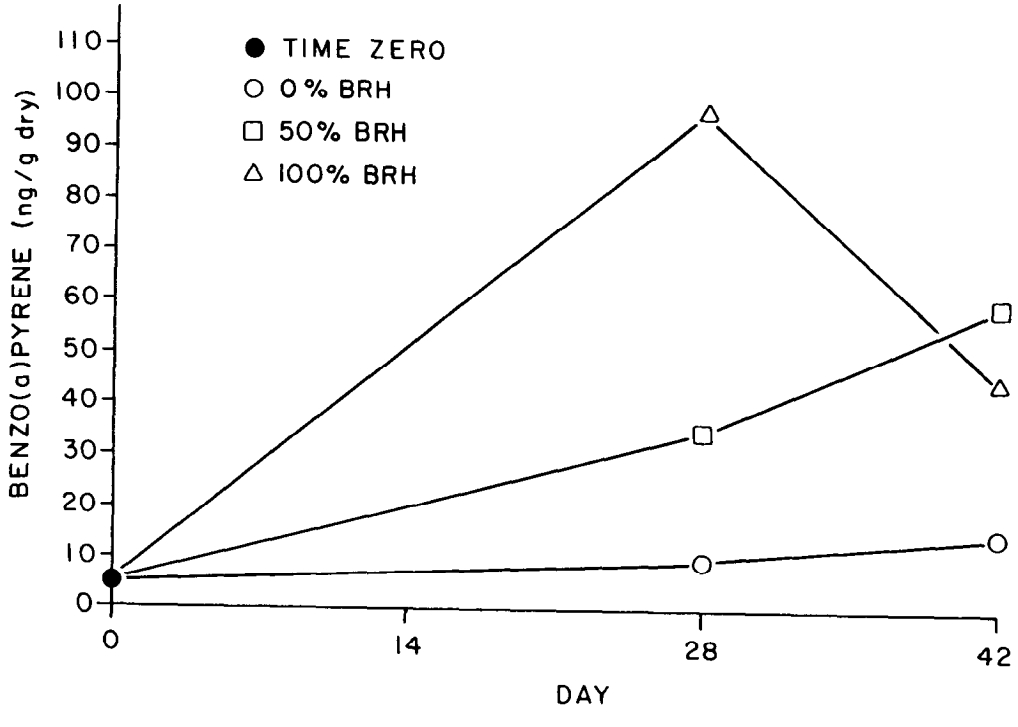


b. 178 alkyl homologs

Figure 7. Concentrations of phenanthrene and 178 alkyl homologs in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

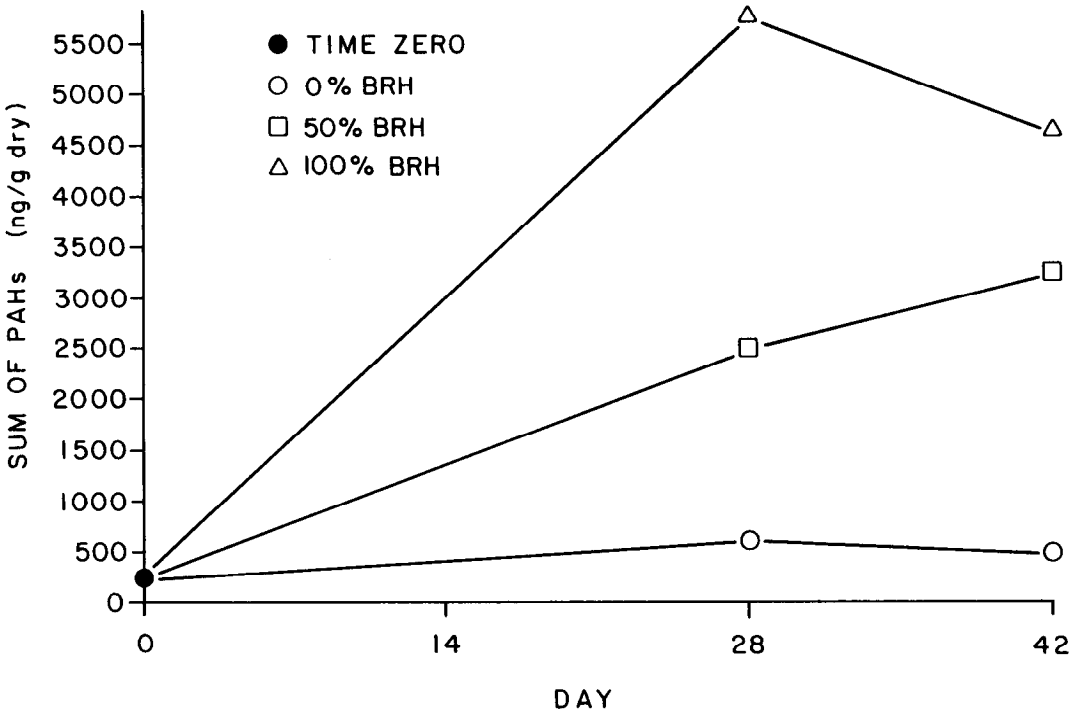


a. Fluoranthene

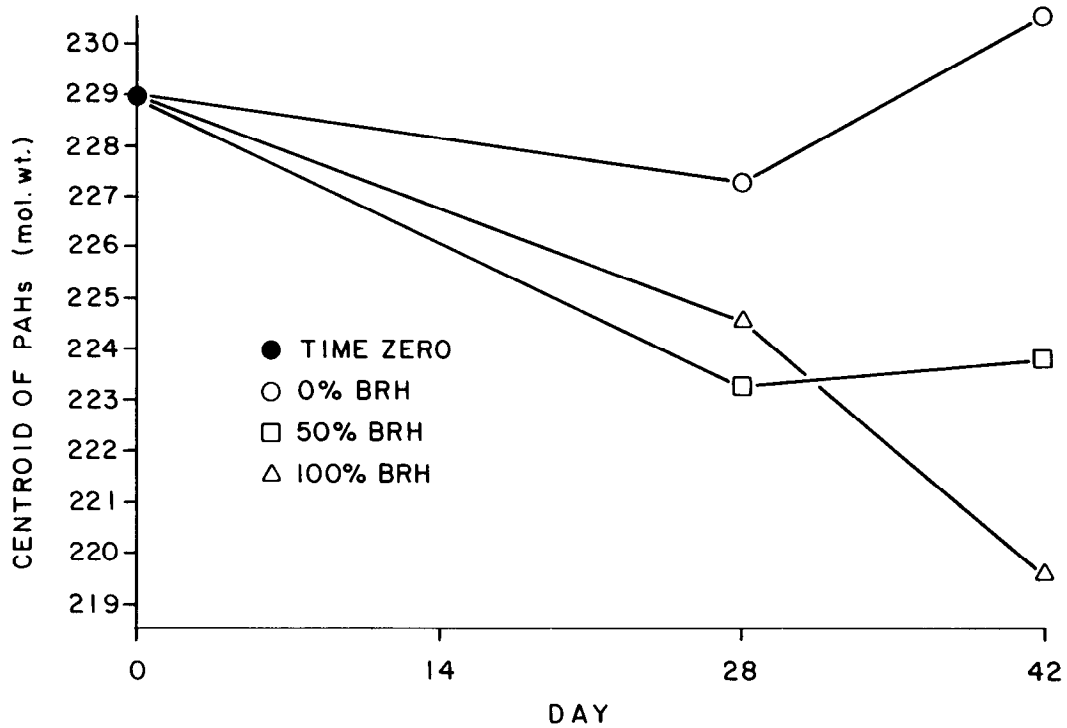


b. Benzo(a)pyrene

Figure 8. Concentrations of fluoranthene and benzo(a)pyrene in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

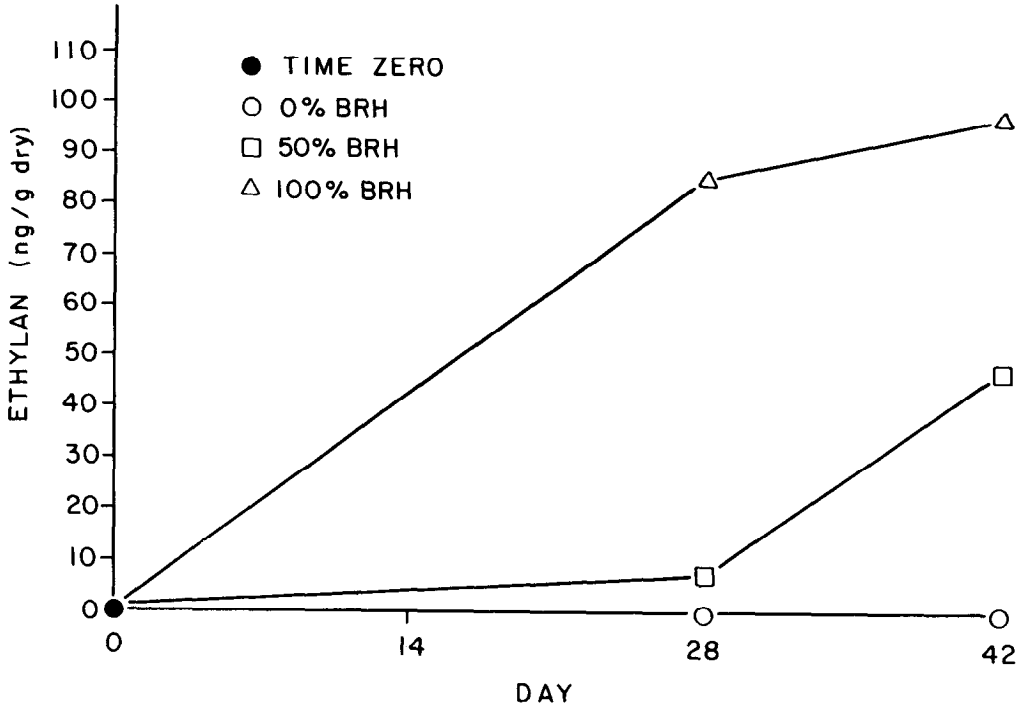


a. SUM of PAHs

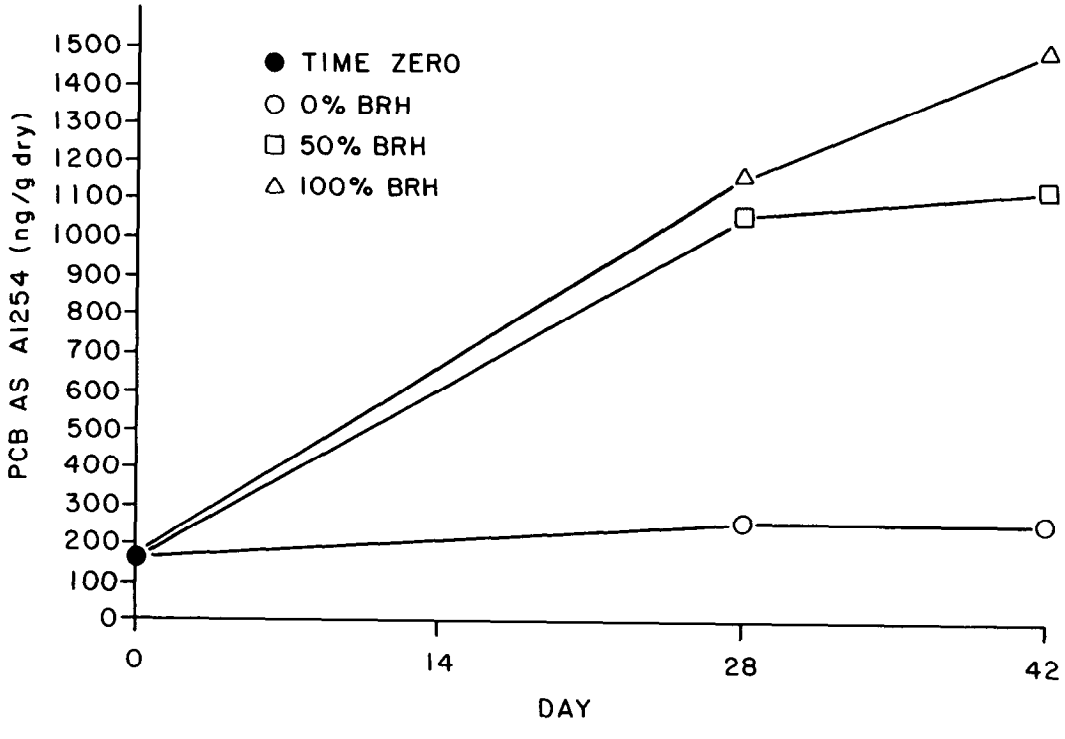


b. CENT of PAHs

Figure 9. Concentrations of the SUM of PAHs and CENT of PAHs in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

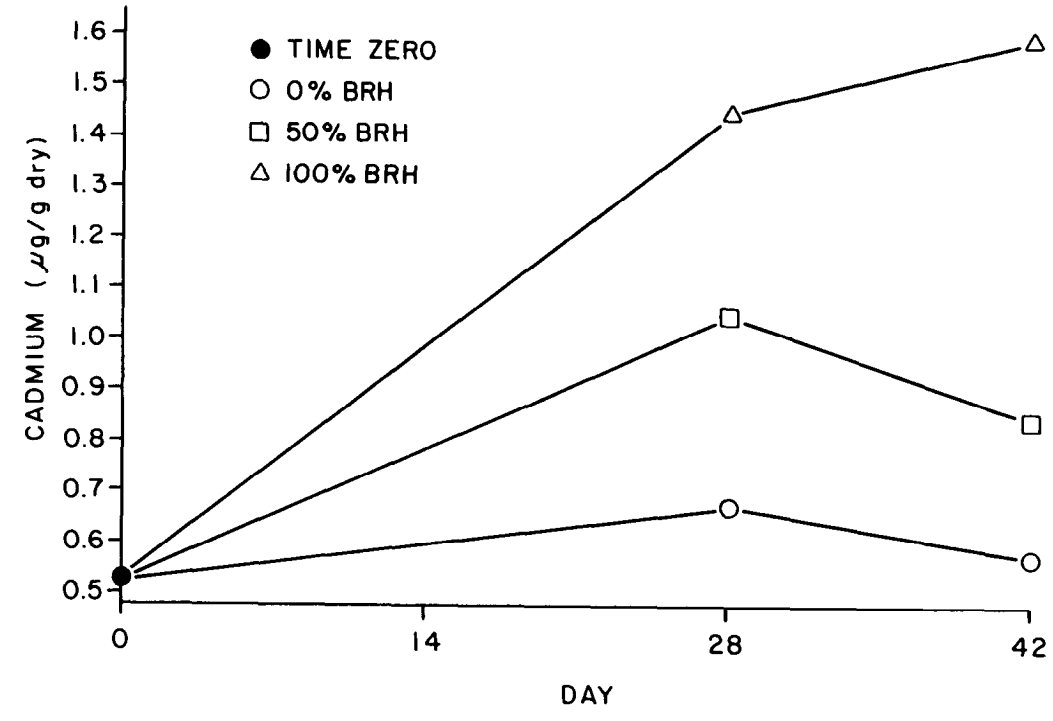


a. Ethylan

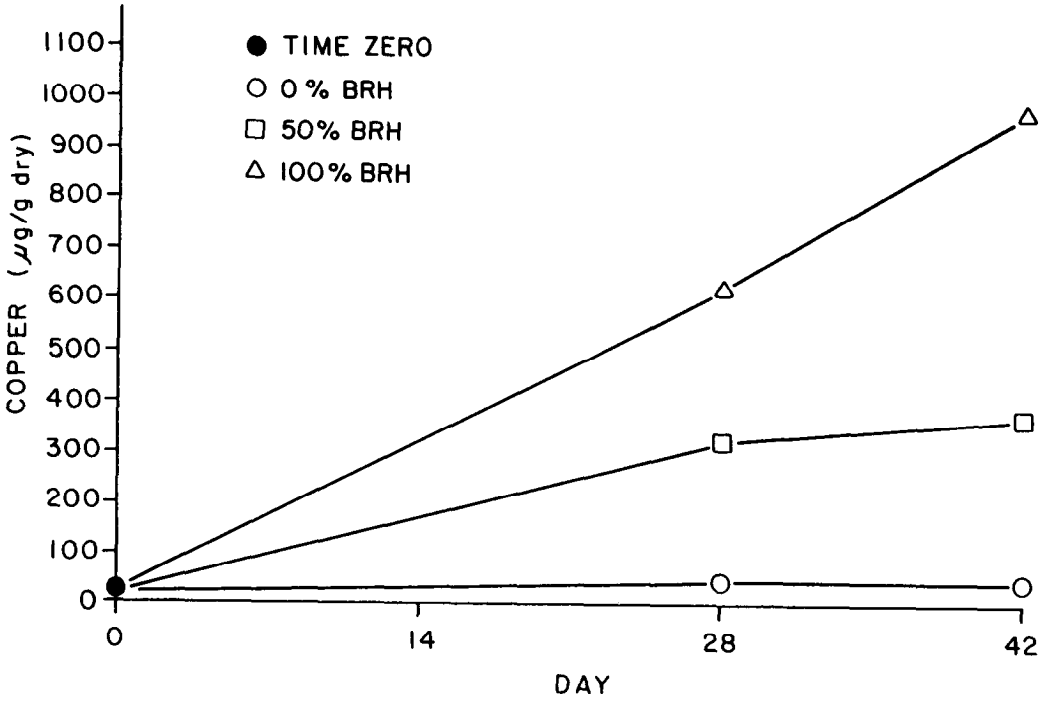


b. PCB as A1254

Figure 10. Concentrations of ethylan and PCB as A1254 in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

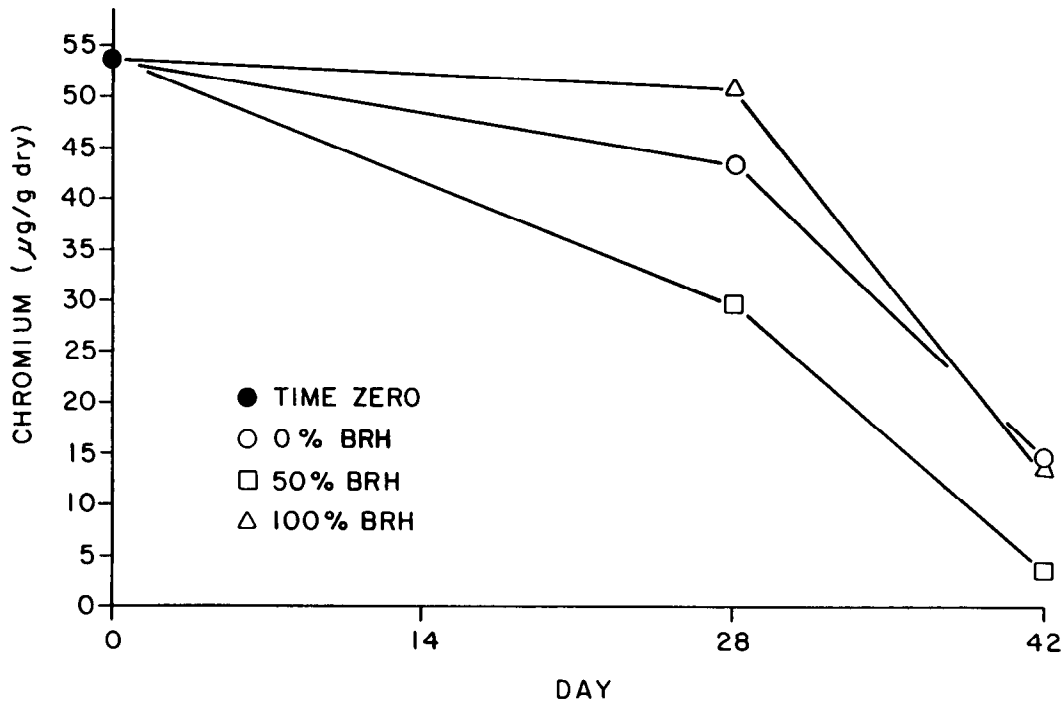


a. Cadmium

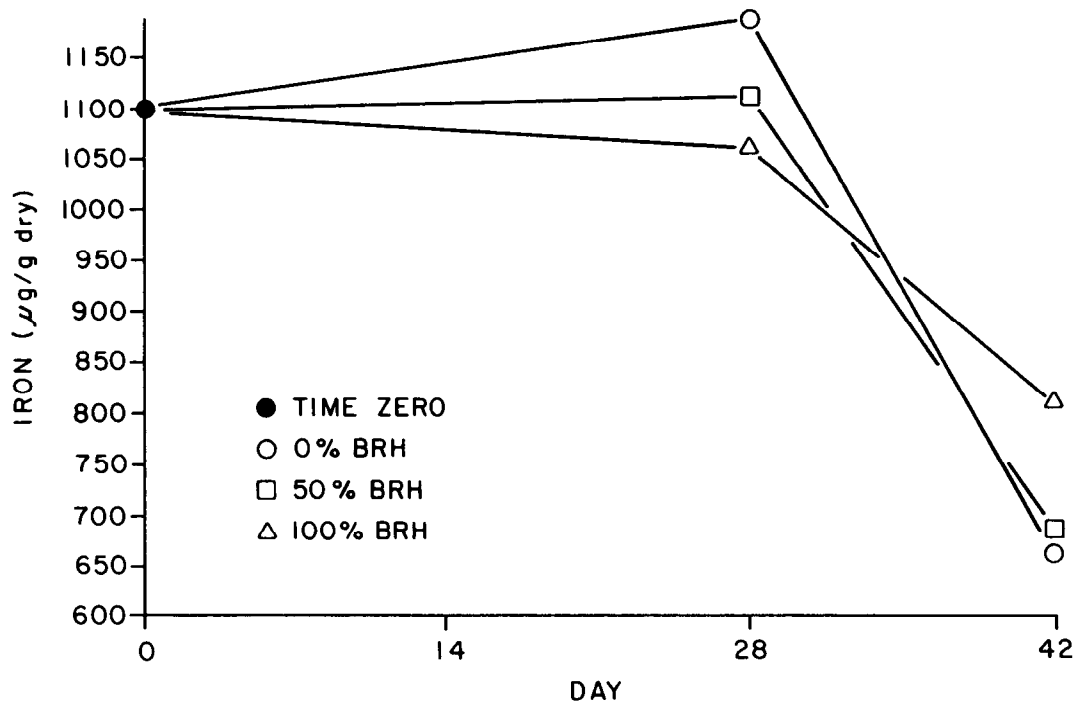


b. Copper

Figure 11. Concentrations of cadmium and copper in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days



a. Chromium



b. Iron

Figure 12. Concentrations of chromium and iron in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

Statistical Properties of the SCE Data

75. This report is based on data from the polychaete *N. incisa*. Because the data base for *N. incisa* is not as extensive as that for another polychaete, *Neanthes arenaceodentata*, the latter data base has been relied upon to determine the need for data transformation in statistical analysis. For clarity, both transformed and untransformed data have been included in all tables.

76. The statistical attributes of SCE data for *N. arenaceodentata* were examined using results from a larval bioassay test (Pesch, Heltshe, and Mueller 1984). This section is based on these data. All baseline observation data were pooled and the variable X = SCE/chromosome was considered. There were 447 metaphase cell observations in this data set. Their frequency distribution was skewed to the right and clearly did not follow a normal distribution. Based upon these results, a transformation of the SCE/chromosome data prior to statistical analysis seemed appropriate. To determine the most appropriate transformation, the mean SCEs/chromosome were plotted for all experimental treatments (63 treatments, 25 observations/treatment) against their standard deviation. There was a very good relationship between standard deviation and mean, further evidence that a transformation of the data was appropriate. Although Latt et al. (1981) mention several possible transformations, it was concluded that the log 10 transformation was appropriate because it removed the relationship between mean and standard deviation; also, means based upon sample sizes as small as five were normally distributed (Pesch, Heltshe, and Mueller 1984). Similar transformations were conducted for the *N. incisa* data.

SCE Laboratory Results

77. The particulate phase/solid phase experiment with *N. incisa* was replicated three times using a randomized complete block design. These data are presented in Table 5. In the pooled data, the SCE frequencies for the B/R treatment were significantly higher ($P = 0.053$, approximately 50 percent higher) than any other treatment. There were no significant differences among the other treatments.

Table 5

SCE/Chromosome Response to Particulate Phase/Solid Phase Dosing of *N. incisa*

Treatment	Replicate 1	Replicate 2	Replicate 3	Pooled Data
		Log-Transformed Data		
B/R*	-0.450 ± 0.050**(25)†	-0.414 ± 0.055(12)	-0.194 ± 0.056(19)	-0.355 ± 0.035(56)
B/B	-0.543 ± 0.050(26)	-0.513 ± 0.054(15)	-0.449 ± 0.053(25)	-0.500 ± 0.031(66)
R/B	-0.608 ± 0.056(25)	ND††	-0.403 ± 0.055(24)	-0.508 ± 0.041(49)
R/R	-0.494 ± 0.145(7)	-0.512 ± 0.049(25)	-0.482 ± 0.123(9)	-0.502 ± 0.046(41)
		Untransformed Data		
B/R	0.306 ± 0.038(25)	0.321 ± 0.053(12)	0.627 ± 0.078(19)	0.418 ± 0.033(56)
B/B	0.246 ± 0.050(26)	0.239 ± 0.037(15)	0.319 ± 0.049(25)	0.272 ± 0.028(66)
R/B	0.200 ± 0.039(25)	ND	0.376 ± 0.065(24)	0.286 ± 0.038(49)
R/R	0.323 ± 0.105(7)	0.252 ± 0.034(25)	0.343 ± 0.115(9)	0.284 ± 0.037(41)

Note: Data expressed as log 10 of means.

* R = reference sediment, B = BRH sediment, numerator = suspended phase, denominator = bedded phase.

** Standard error of the mean.

† Number in parentheses is sample size (N).

†† ND = no data.

78. Johns and Gutjahr-Gobell (1985) studied the impact of BRH sediment on the bioenergetics of *N. incisa*. These studies were conducted simultaneously in the same exposure system as the SCE studies. Data for net growth efficiency and SCE are compared in Figure 13. They found that the presumably worst case treatment (B/B) caused *N. incisa* to lose weight during the experiment, whereas the B/R treatment was not significantly different from the control treatment (R/R) for any of the measures of energetic effects. The worms in the worst case treatment (B/B) were inactive, did not eat, and had

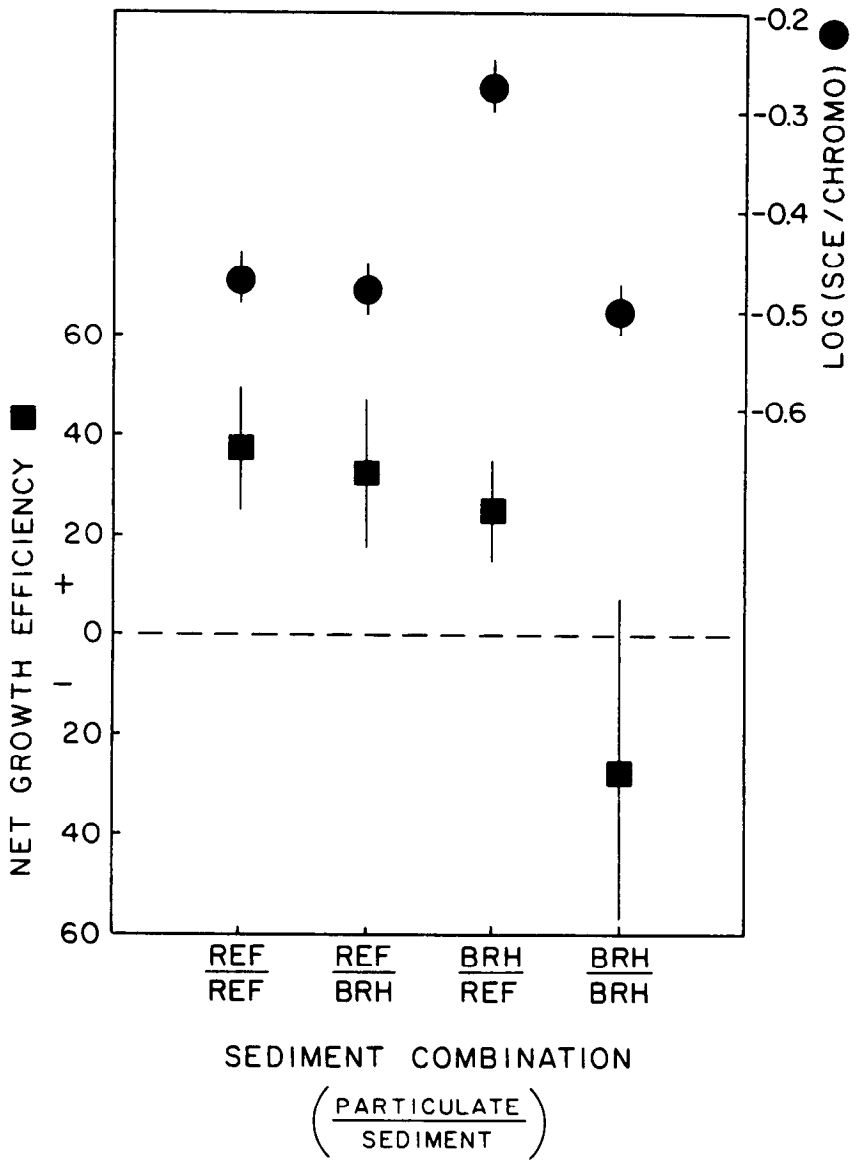


Figure 13. A comparison of SCE and net growth efficiency responses measured in the same experiments suggesting the dependence of the SCE response on the physiological condition of the worms

reduced metabolic activity. These responses, in effect, isolated these worms from exposure. The worms in the B/R treatment did well metabolically and thus were susceptible to the BRH exposure. The worms in the R/B treatment physically isolated themselves from the exposure by moving from the BRH bedded phase sediment into REF sediment deposited within the exposure chamber during the experiments.

Field Results

Exposure

79. *Nephtys incisa* exposure estimated from tissue residues. The first method used to estimate exposure conditions of *N. incisa* to BRH material in CLIS involved the laboratory-generated relationships between PCB tissue residues and BRH exposures. Using this relationship and the PCB tissue residues in field-collected *N. incisa*, estimates of field BRH exposure concentrations were calculated. There are several assumptions in this approach: *N. incisa* provides an integrated measure of exposure; *N. incisa* tissue residues were at steady-state with BRH exposure concentrations at the time of sampling; and PCBs are a good chemical marker for BRH sediments. Based on the results of the laboratory experiment, each of these assumptions seems reasonable.

80. The estimated exposures resulting from this approach are presented as milligrams per litre BRH for each station and collection date in Table 6. *Nephtys incisa* at CNTR were buried during disposal of the dredged material and did not recolonize the dredged material mound until the spring of 1984. When the worms recolonized the mound, sampling began. The data in Table 6 display clear spatial and temporal trends. The highest estimates were in the immediate vicinity of the disposed BRH material (400E) during the summer of 1983. There was a decrease in exposure at 400E and 1000E in 1984 and 1985.

81. *Nephtys incisa* exposure estimated from physical data. Benthic exposure at the FVP disposal site can occur through both the suspended and bedded sediments. This section describes predictions of the maximum upper bound suspended sediment concentrations from 1 m above the bottom to the sediment-water interface. This calculation is based upon the assumption that the suspended solids at the sediment-water interface consist totally of BRH sediment and that the contaminant concentrations are similar to those found in the dredged material prior to disposal.

Table 6

Estimated Concentrations of BRH Sediment (mg/ℓ) Suspended at the Sediment-Water Interface Based on PCB Concentrations in Field-Collected *N. incisa*

Date	Station			
	CNTR	400E	1000E	REFS
17 Apr 82	-	0	-	0
16 Nov 82	-	0	-	2
16 Feb 83	-	9	-	3
12 Apr 83	-	15	-	8
02 Jun 83	-	95	43	2
03 Jul 83	-	114	44	2
06 Sep 83	-	131	88	12
29 Nov 83	-	51	26	0
20 Mar 84	47	38	10	0
16 Oct 84	53	29	10	3
24 Jan 86	76	5	4	0

82. Total suspended solids concentrations were measured at the FVP site at 1 m above the sediment-water interface with an in-situ monitoring platform (Bohlen and Winnick 1986). Although there is considerable variation in the data through one tidal cycle, average background suspended solids were estimated to be 10 mg/ℓ, while during typical storm conditions suspended solids concentrations averaged 30 mg/ℓ for periods of 4 to 7 days (Munns et al. 1986).

83. Using an acoustic profilometer, the suspended sediment concentrations at 1 m above the bottom were found to increase exponentially to the sediment-water interface. The upper and lower limits for this increase are 10× and 1×, respectively, depending on hydrographic conditions (Bohlen and Winnick 1986). These data, in conjunction with suspended sediment data for 1 m above the bottom, can be used to predict the suspended solids concentrations at the sediment-water interface.

84. For example, the suspended solids concentration under background conditions (10 mg/ℓ) would increase to 100 mg/ℓ for the 10× enrichment at the sediment-water interface, and decrease to 10 mg/ℓ for the quiescent conditions. Likewise, under storm conditions (30 mg/ℓ), sediment-water interface suspended solids concentrations would range from 300 to 30 mg/ℓ for the 10× and 1× enrichments, respectively (Figure 14). These conditions represent the maximum upper bound exposures that would be expected to occur at the sediment-water interface and could be made using predisposal, site characterization data.

85. A more probable estimate is provided by using contaminant concentrations present in the sediments after disposal. It is this material that will be resuspended and transported as suspended solids to populations outside

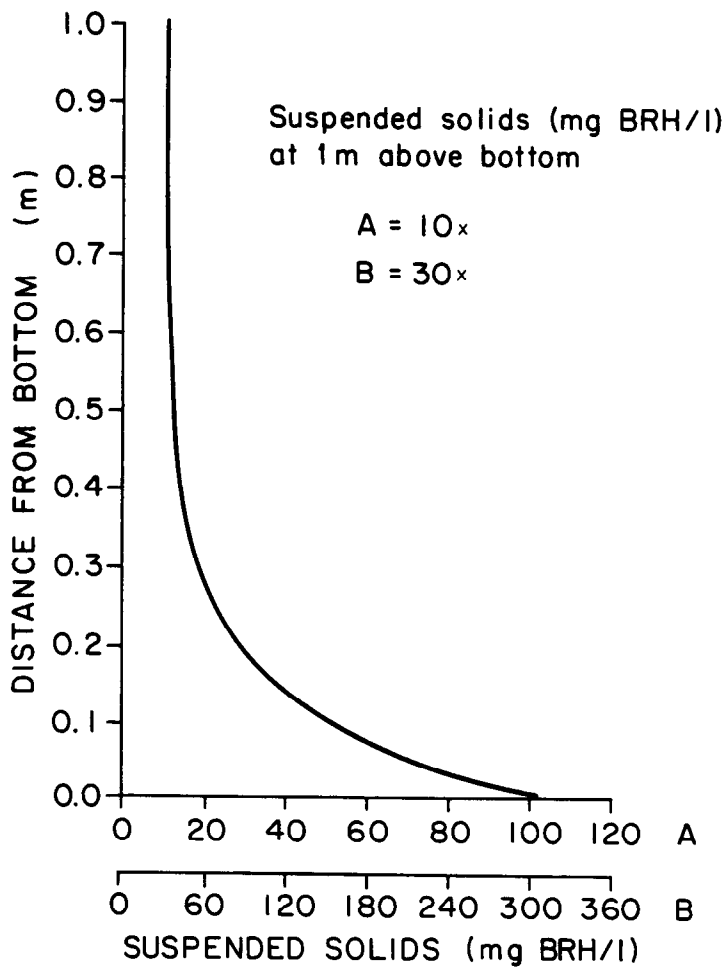


Figure 14. Suspended sediment concentrations from 1 m above the bottom to the sediment-water interface for storm and background conditions

the disposal site. Assuming that resuspended surficial sediments are the source of contaminants for the suspended sediments, the maximum upper bound estimates can be adjusted to reflect the spatial and temporal changes in contaminant concentration. These changes are represented as percentages of BRH sediment in the 0- to 2-cm surface layer at CNTR, 200E, 400E, and 1000E from June 1983, immediately after disposal, to October 1985 (Table 7). The combination of these percentages and the total suspended solids concentrations predicted for the sediment-water interface results in concentrations of BRH suspended sediments at the sediment-water interface for each station and sampling date (Table 8).

86. The sediment samples used for the percent calculations were not replicated; therefore, no variability estimates are available. However, certain trends in the data are evident (Table 7). The percentages of BRH sediment (<50 percent) at CNTR and 200E were low compared with the barrel sediments collected predisposal. There is a gradient of BRH material that is a function of both distance from the center of the mound and of time from

Table 7

Percent BRH Sediment in the Surficial Sediments at the FVP Disposal Site

Date	Station			
	CNTR	200E	400E	1000E
Jun 83	44.5	41.1	12.5	1.8
Jul 83	15.0	37.4	3.3	1.6
Sep 83	32.0	36.7	4.9	2.0
Dec 83	32.8	36.1	9.5	4.4
Mar 84	4.4	2.2	1.9	1.8
Jun 84	9.5	15.6	0.5	0.7
Sep 84	10.0	0.8	3.5	0.5
Oct 84	2.6	--	0.2	1.6
Dec 84	35.1	11.3	0.0	1.0
Oct 85	0.2	21.0	0.0	0.0

Table 8

Concentration of BRH (mg/ℓ) at the Sediment-Water Interface for Total Suspended Sediment Concentrations of 30 mg/ℓ and 10 mg/ℓ and an Enrichment of 10×*

	Station							
	CNTR		200E		400E		1000E	
Date	300	100	300	100	300	100	300	100
Jun 83	133.5	44.5	123.3	41.1	37.5	12.5	5.4	1.8
Jul 83	45.0	15.0	112.2	37.4	9.9	3.3	4.8	1.6
Sep 83	96.0	32.0	110.1	36.7	14.7	4.9	6.0	2.0
Dec 83	98.4	32.8	108.3	36.1	28.5	9.5	13.2	4.4
Mar 84	14.2	4.4	6.6	2.2	4.7	1.9	5.4	1.8
Jun 84	28.5	9.5	46.8	15.6	1.5	0.5	2.1	0.7
Sep 84	30.0	10.0	2.4	0.8	10.5	3.5	1.5	0.5
Oct 84	7.8	2.6	--	--	0.6	0.2	4.8	1.6
Dec 84	105.3	35.1	33.9	11.3	0	0	3.0	1.0
Oct 85	0.6	0.2	63.0	21.0	0	0	0	0

* BRH concentrations for the 1× enrichment can be obtained by dividing the tabular values by 10.

disposal. BRH sediment concentrations were highest at CNTR and 200E immediately after disposal, and decreased significantly through October 1984. Concentrations were elevated in December 1984 at CNTR and 200E and again in October 1985 at 200E. The BRH concentrations at 400E also decreased through time and, after December 1983, were the same as those at 1000E.

87. The 1- to 2-percent BRH sediment calculated for 1000E represents a quantitatively measured elevation above background and is supported by tissue residue data for *N. incisa*. This contamination could have resulted from the dispersion of dredged material during disposal, the errant disposal of BRH material in the vicinity of 1000E, or the continuous transport of contaminated material from the disposal mound.

88. The estimates of exposure to BRH material at the sediment-water interface derived from tissue concentrations of PCB and from the maximum upper

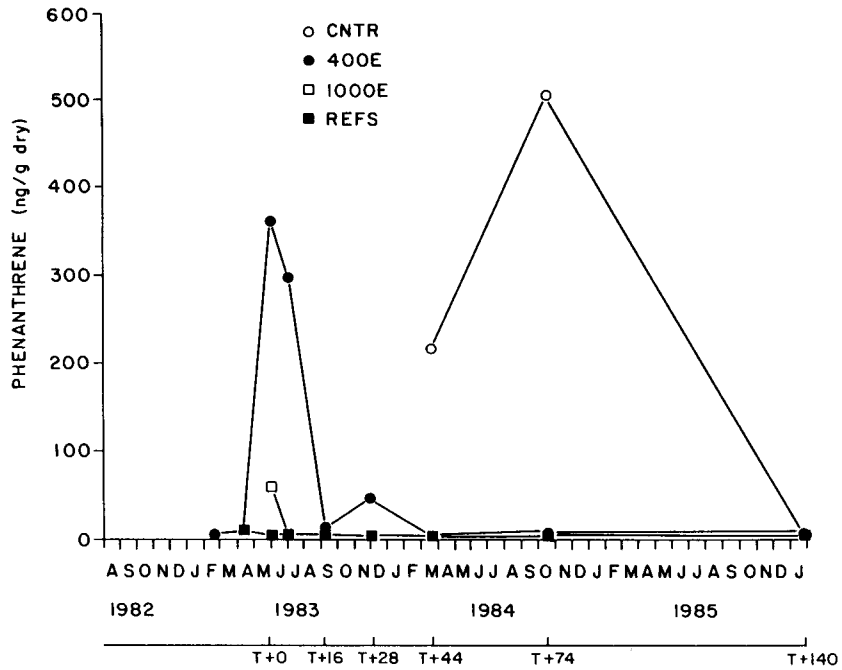
bound predictions agreed well. The exposure estimates based on the chemistry of the 0- to 2-cm surface sediments were low. If the exposure estimates based on tissue concentrations of PCB are accepted as a valid check on the exposure estimates from the physical data, it is concluded that the higher estimates of exposure are accurate. The simplest explanation is that the 0- to 2-cm sampling procedure integrates clean and contaminated sediment, thus underestimating the actual exposures experienced by the worms. The data suggest that the worms were exposed to a thin, surface layer of contaminated sediment.

Tissue residues

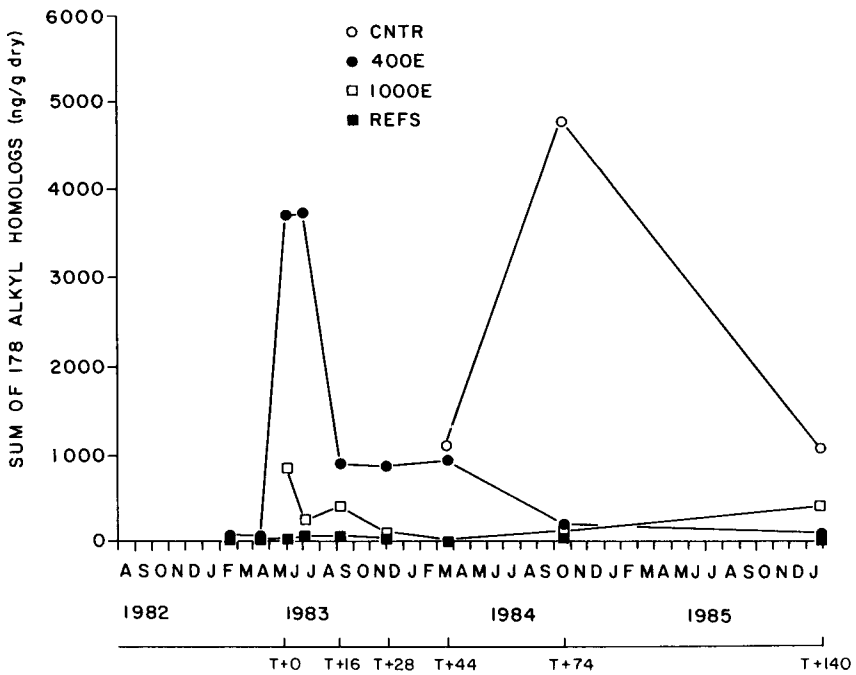
89. The tissue concentrations for the *N. incisa* collected at the CLIS site during the FVP study are presented graphically for each of the 12 selected chemical variables in Figures 15-20. The raw data shown on these figures are included in the Appendix Tables B3-B17.

90. Clear spatial and temporal patterns of tissue concentrations of PCBs and PAHs were found. Highest tissue concentrations were determined at station 400E with lowest concentrations at station REFS. When *N. incisa* recolonized the dredged material site at station CNTR in the spring of 1984, the tissue concentrations of PCBs in these worms were comparable with those found at 400E immediately postdisposal.

91. The temporal patterns of the field tissue residues show a rapid increase in organic residue values during and immediately postdisposal at 400E and at 1000E. The PAH residues for *N. incisa* showed an increase immediately postdisposal. This was followed by a rapid decline during July and August. The phenanthrene residue value returned to background levels by September, but the higher molecular weight PAH tissue residues tended to remain at approximately 25 percent of their maximum values for an additional year. The PCB residues at 400E increased rapidly immediately after disposal and, unlike the PAHs, remained elevated through September and declined only 50 percent by December 1983. Unlike the PAHs, PCB residues increased 2.5 times REFS at 1000E postdisposal and remained elevated above REFS until October 1984. There were no clear temporal or spatial patterns for inorganic tissue residues for *N. incisa* from the field.

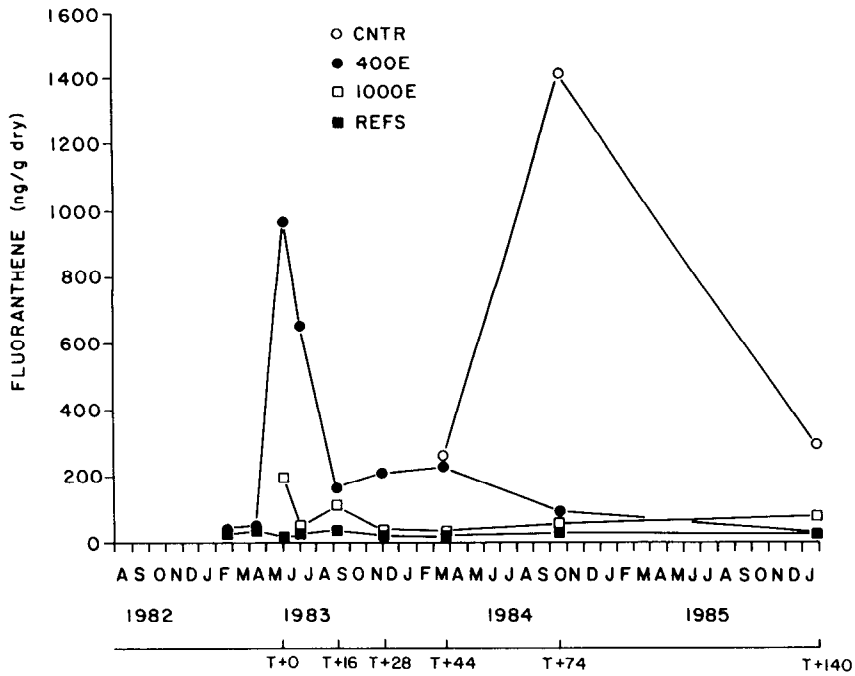


a. Phenanthrene

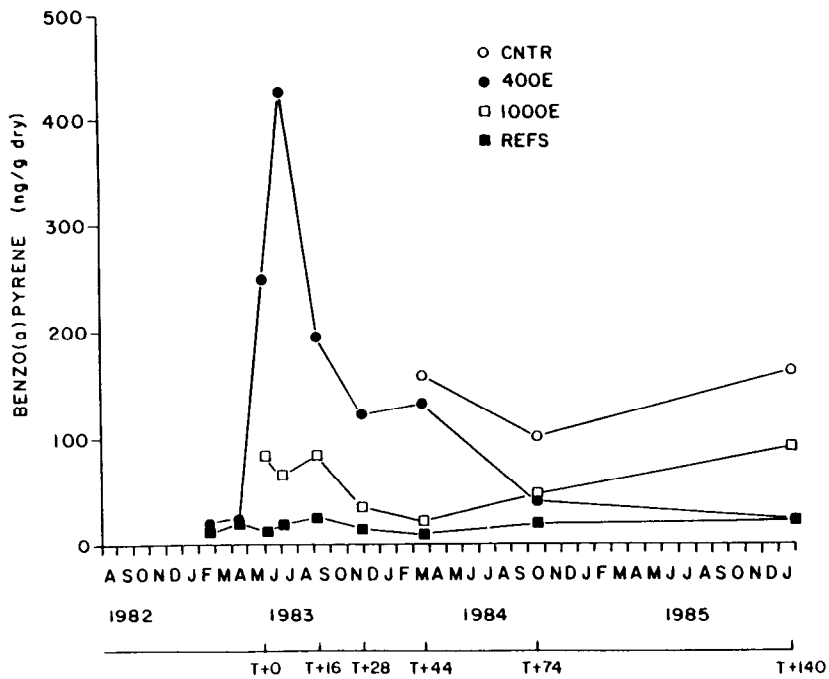


b. Sum of 178 alkyl homologs

Figure 15. Concentrations of phenanthrene and the 178 alkyl homologs in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

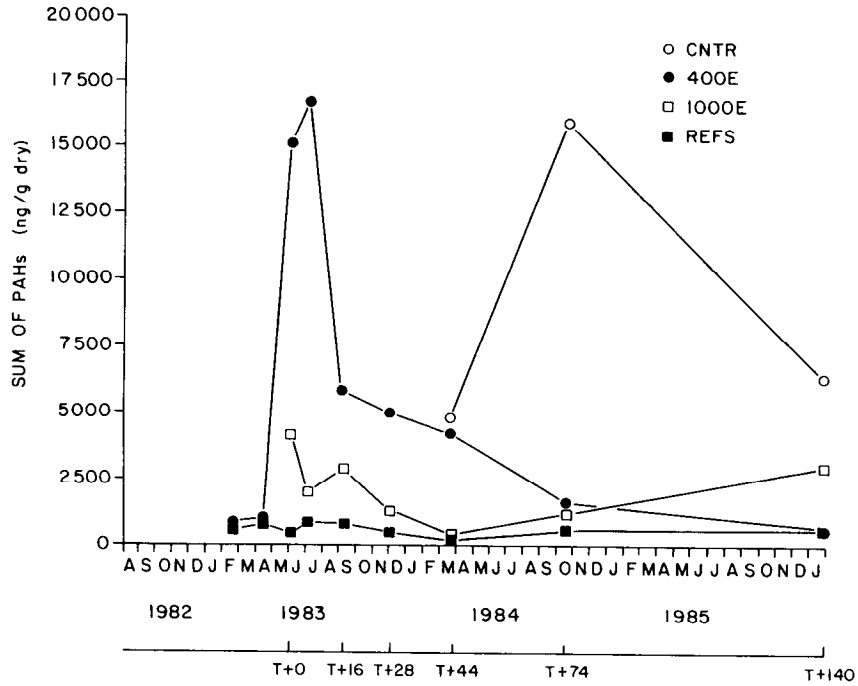


a. Fluoranthene

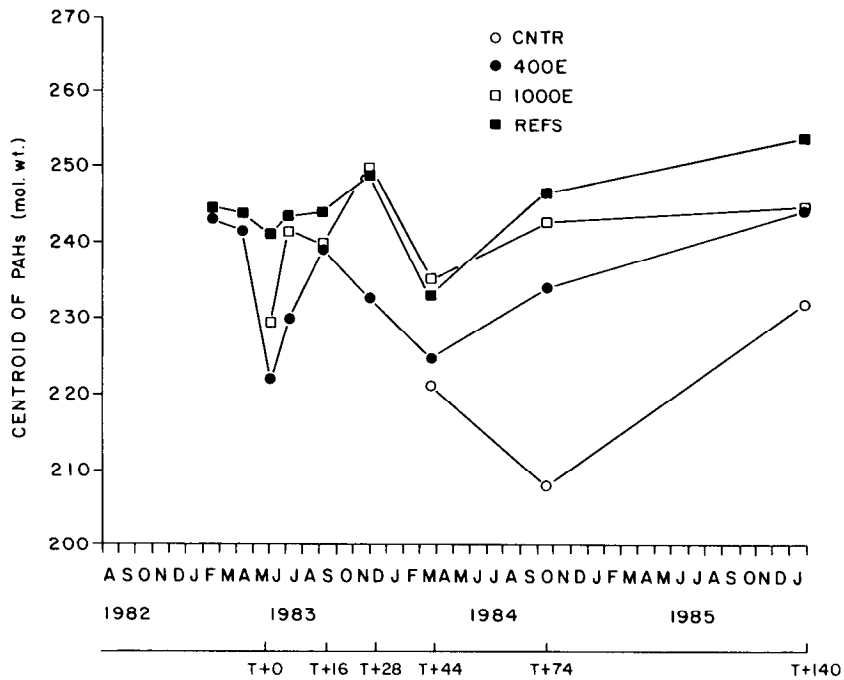


b. Benzo(a)pyrene

Figure 16. Concentrations of fluoranthene and benzo(a)pyrene in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

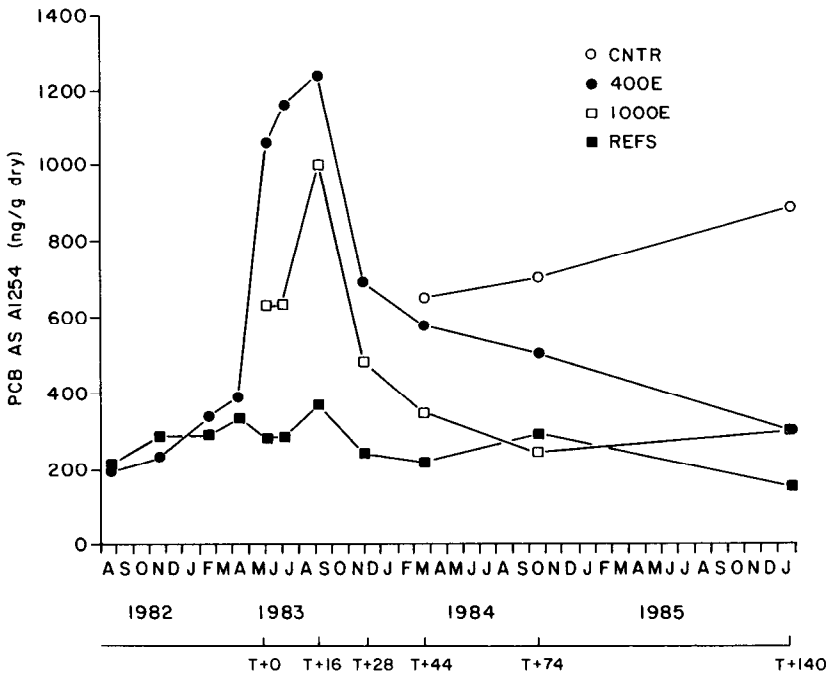


a. SUM of PAHs

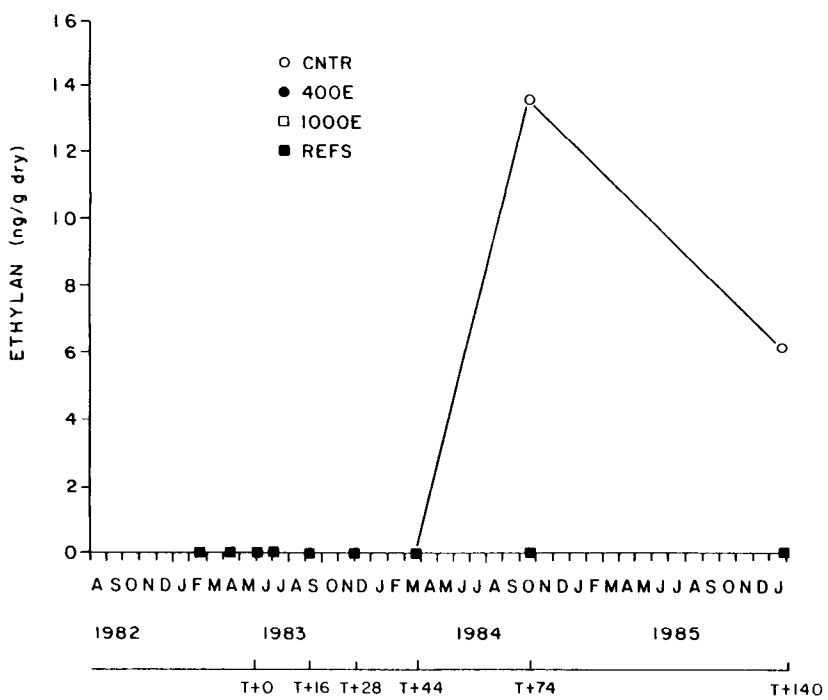


b. CENT of PAHs

Figure 17. Concentrations of the SUM of PAHs and CENT of PAHs in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

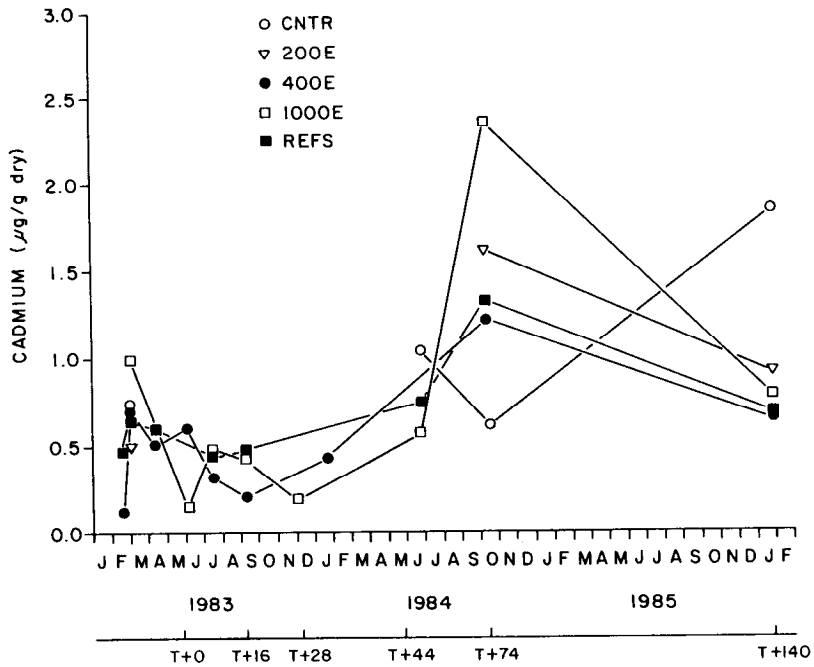


a. PCBs as A1254

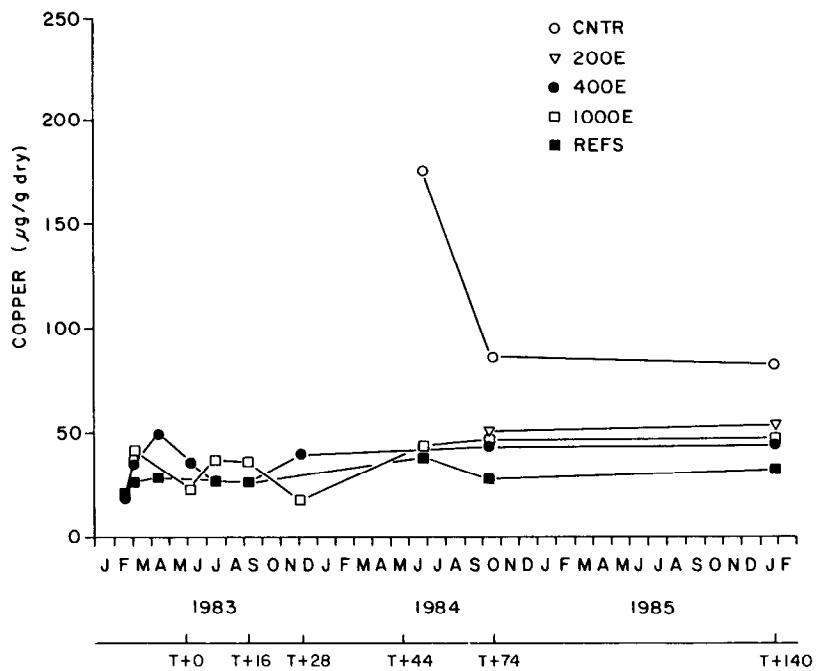


b. Ethylan

Figure 18. Concentrations of PCBs as A1254 and ethylan in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

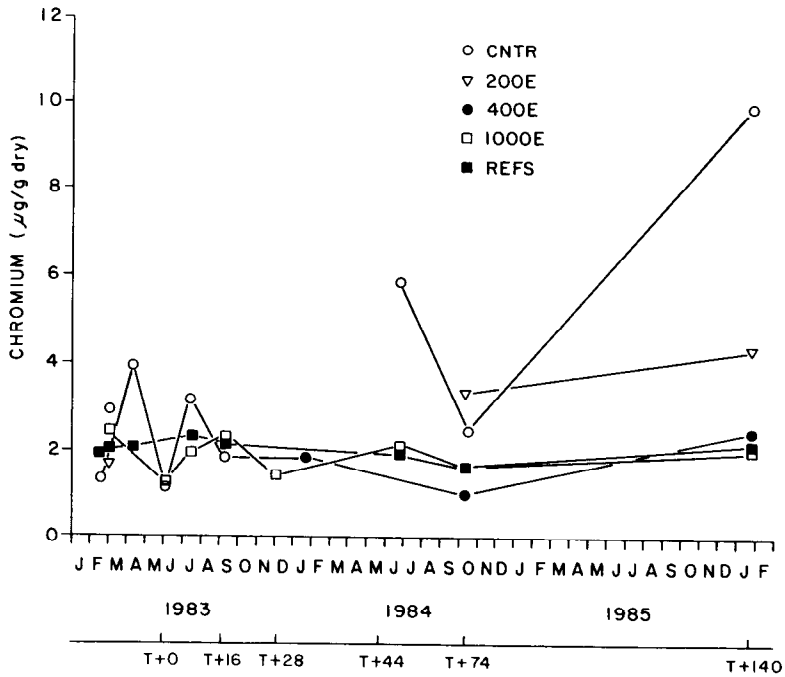


a. Cadmium

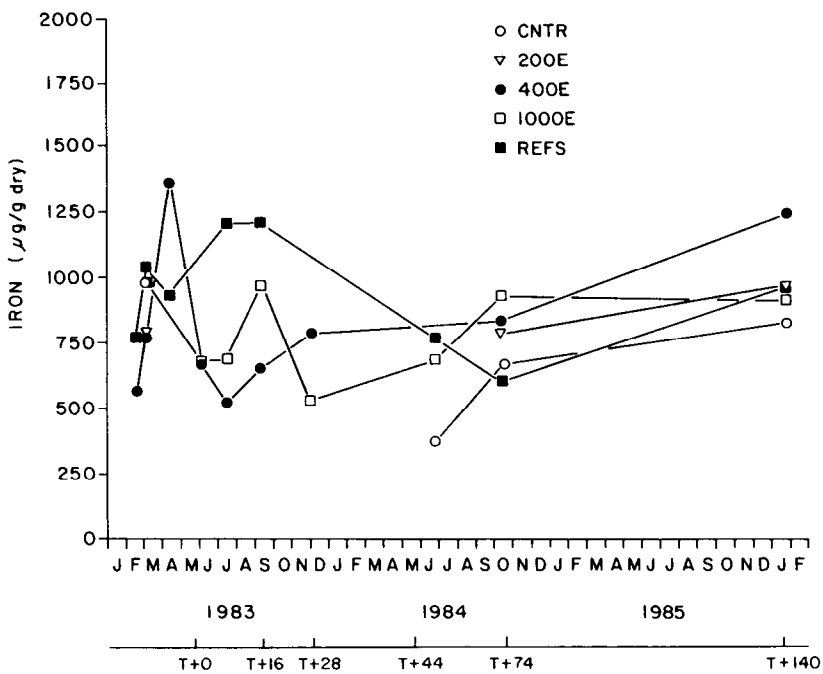


b. Copper

Figure 19. Concentrations of cadmium and copper in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates



a. Chromium



b. Iron

Figure 20. Concentrations of chromium and iron in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

SCE Field Results

92. SCE observations were made on juvenile (young of the year) *N. incisa* collected at stations 200E, 400E, 1000E, and REFS (Figure 2). Data are available for three sampling dates: 6 June 1983 (T + 3 weeks), 26 August 1983 (T + 14 weeks), and 13 December 1983 (T + 28 weeks). These data are summarized in Table 9 and presented graphically in Figure 21. Two patterns are clear. First, the SCE response is low in June, rises significantly in August, and declines in December. Second, there are no differences among stations on any given date. The pattern of increase in the August data is consistent at all stations. During the cruise for the August field survey, worms were collected for a laboratory replicate test. These worms, collected at the REFS site, were used for replicate 3 in Table 5. These data are compared graphically in Figure 22.

Table 9

SCE/Chromosome Response in *N. incisa* Sampled at the FVP Biological Effects Stations in CLIS

Station	Sampling Time Postdisposal (weeks) June 1983 T + 3 weeks	August 1983 T + 14 weeks	December 1983 T + 28 weeks
	Log-Transformed Data		
200E	-0.749 ± 0.029*(2)**	-0.252 ± 0.066(12)	-0.404 ± 0.205(4)
400E	-0.603 ± 0.029(2)	-0.203 ± 0.049(15)	-0.483 ± 0.080(7)
1000E	-0.705 ± 0.295(3)	-0.169 ± 0.049(25)	-0.337 ± 0.079(5)
REFS	-0.603 ± 0.054(4)	-0.200 ± 0.050(14)	-0.499 ± 0.053(12)
	Untransformed Data		
200E	0.079 ± 0.012(2)	0.532 ± 0.092(12)	0.406 ± 0.156(4)
400E	0.150 ± 0.017(2)	0.582 ± 0.074(15)	0.265 ± 0.073(7)
1000E	0.222 ± 0.222(3)	0.658 ± 0.060(25)	0.391 ± 0.088(5)
REFS	0.155 ± 0.029(4)	0.584 ± 0.074(14)	0.247 ± 0.052(12)

Note: Data expressed as log 10 of means.
* Standard error of the mean.
** Number in parentheses is sample size (N).

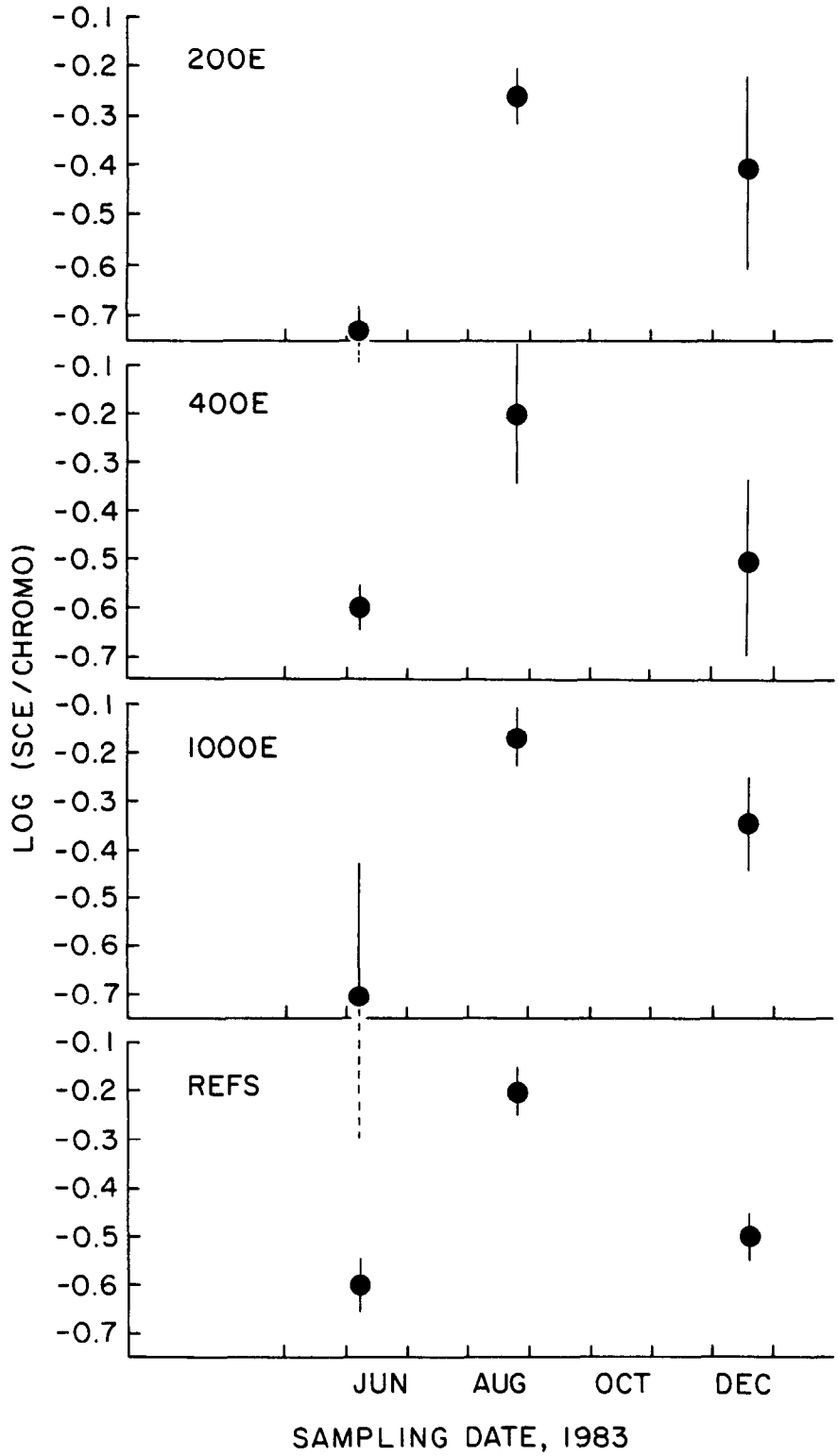


Figure 21. SCE response measured in *N. incisa* sampled at the FVP field stations following disposal of BRH sediment. There were no differences among stations on any date

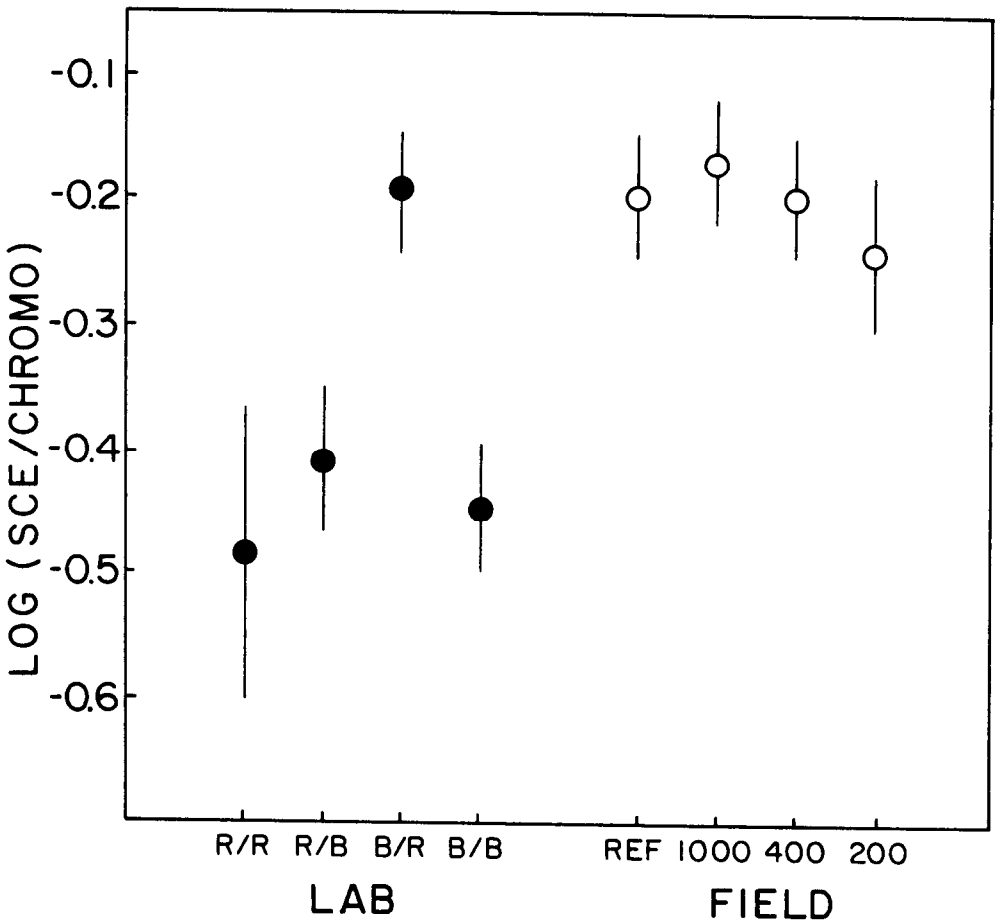


Figure 22. Comparison of laboratory and field data for SCE response in *N. incisa*. Worms for the laboratory test were collected at the south reference station during the cruise for the field observations (14 weeks postdisposal). All field data were significantly higher (2×) than laboratory controls (R/R). The laboratory B/R treatment data were the same as all the field data

Laboratory-to-Field Comparisons

93. The laboratory-to-field comparison was completed in two parts and included both tissue residue and effects data. The approach taken was to first establish whether exposure conditions were similar in the laboratory and the field by comparing laboratory and field residue data. Comparable tissue residues were interpreted as being indicative of comparable BRH exposures. The second step was to compare the SCE values of the laboratory and field worms.

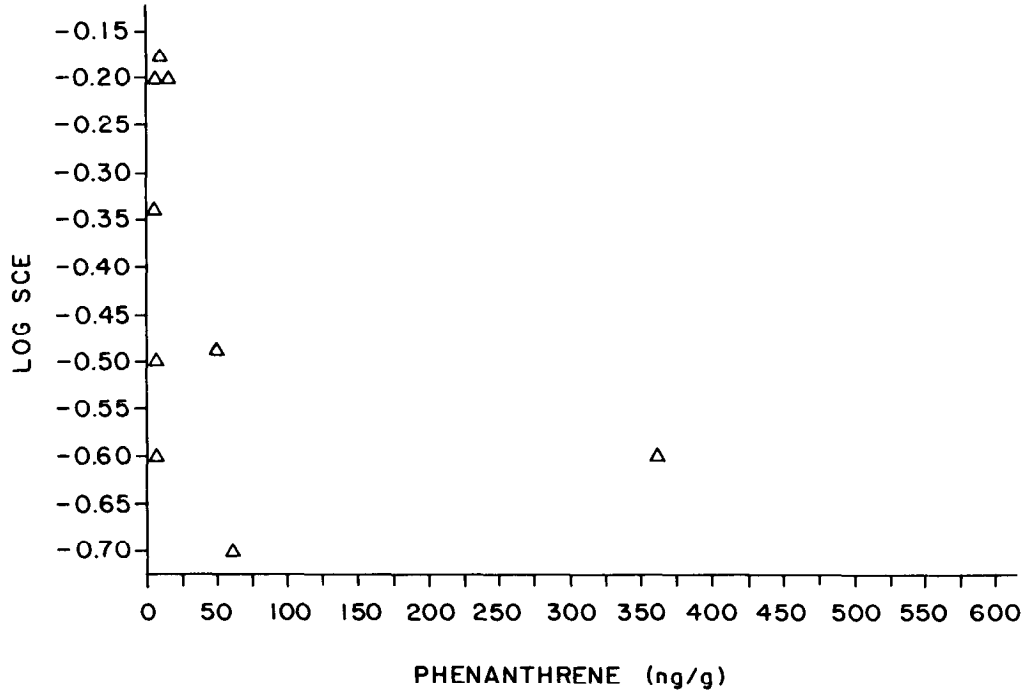
94. Data for the 12 representative chemical variables were analyzed statistically by cluster analysis. The purpose of this procedure was to identify distinctive patterns of association among the *N. incisa* sampled from both laboratory experiments and field stations. The cluster analysis revealed no consistent clustering of the laboratory data separate from field data. This agrees with the overlapping range of residue data in laboratory and field samples. The implication is that laboratory exposures to BRH material accurately reflected the range of field exposures to BRH material for *N. incisa*.

95. The SCE response data were the same statistically for the control treatments (R/R) in all three laboratory replicate tests. The SCE data for the 6 June 1983 (T + 3 weeks) field survey were statistically the same for all four stations, and these data did not differ significantly from the SCE data for the laboratory control treatments. The SCE data for the 12 December 1983 (T + 28 weeks) field survey were statistically the same for all four stations, and these data did not differ significantly from the data for the laboratory control treatments or from the data for the 6 June 1983 field survey. The SCE data for the 26 August 1983 (T + 14 weeks) field survey were statistically the same for all four stations, and these data were significantly higher than both the field data for June and December and the laboratory control (R/R) data. The laboratory B/R treatment SCE data from the third replicate test were the same as the SCE data for the four field stations sampled on 26 August 1983.

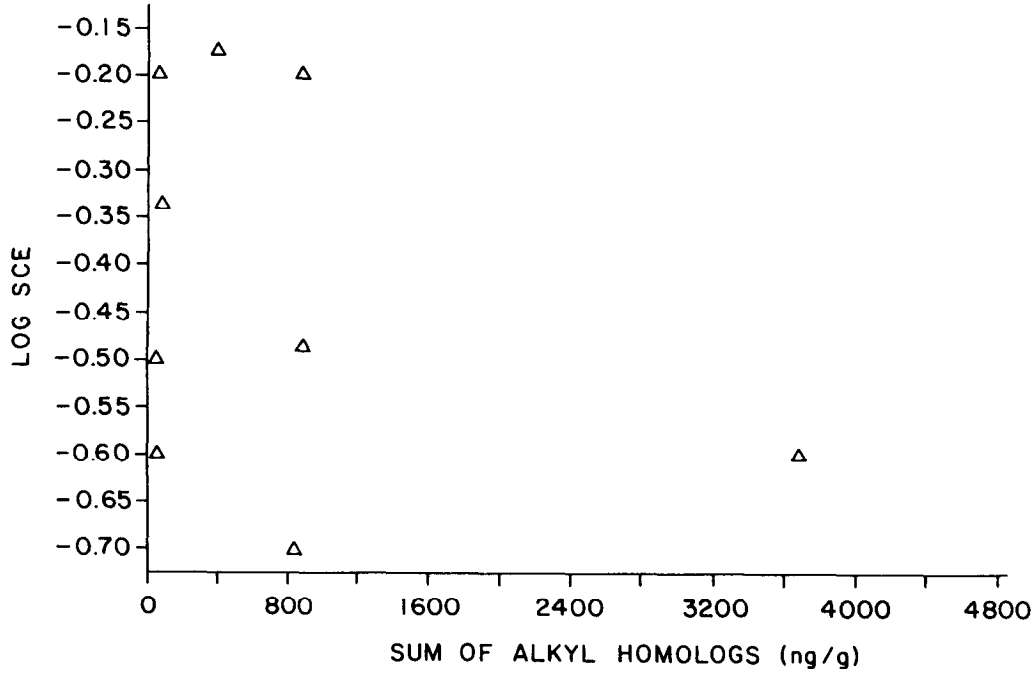
Residue-Effects Comparisons

96. Regression analysis was used to determine whether any statistical relationship existed between the SCE values and the tissue residues. These comparisons are limited to data from stations 400E, 1000E, and REFS for June, September, and December 1983. There were no other field samples or laboratory experiments where chemical analysis and SCE observations were measured successfully simultaneously. The relationship between SCE and tissue residues for each of the selected 10 chemicals, and two summary statistics, are presented graphically in Figures 23-28. The presence of a regression line on any graph indicates a significant relationship ($P \leq 0.05$) between SCE and the tissue residue for that particular element, compound, or summary statistic.

97. With the exception of chromium, the graphs indicate that there were no relationships between SCE and tissue residues. Small sample sizes

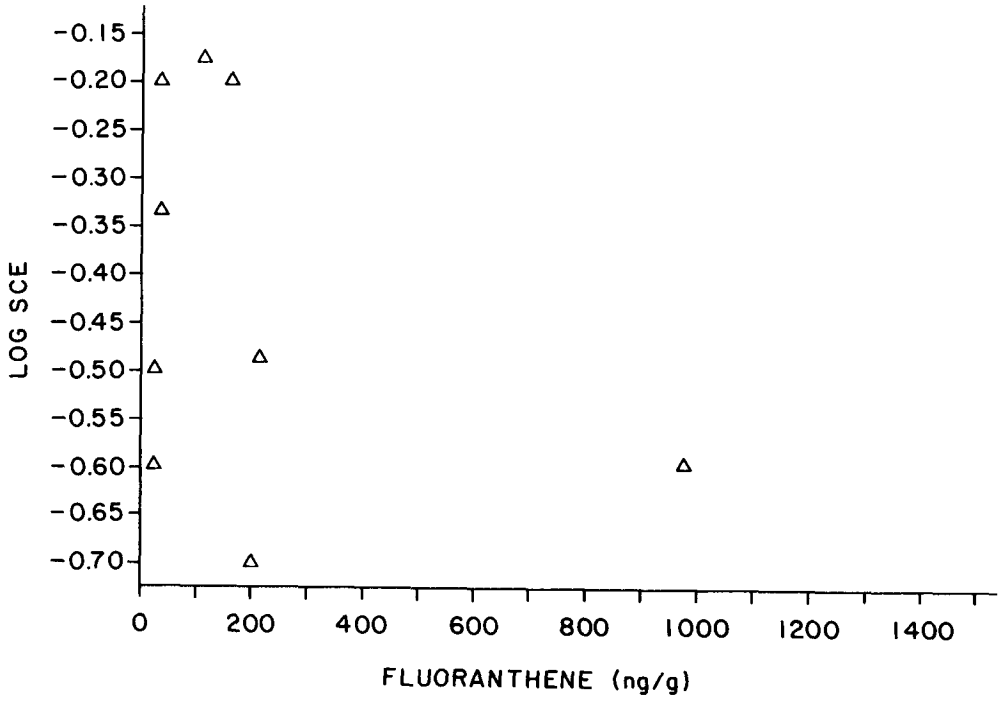


a. Phenanthrene

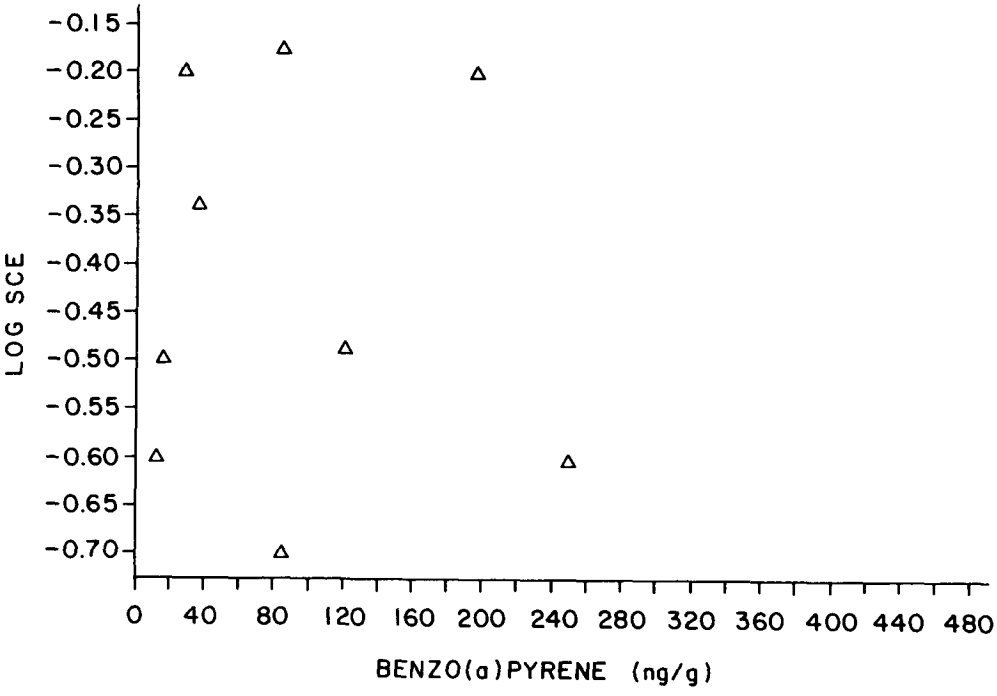


b. Sum of 178 alkyl homologs

Figure 23. Relationship between SCE response and concentrations of phenanthrene and 178 alkyl homologs measured in tissues of field-collected *N. incisa*

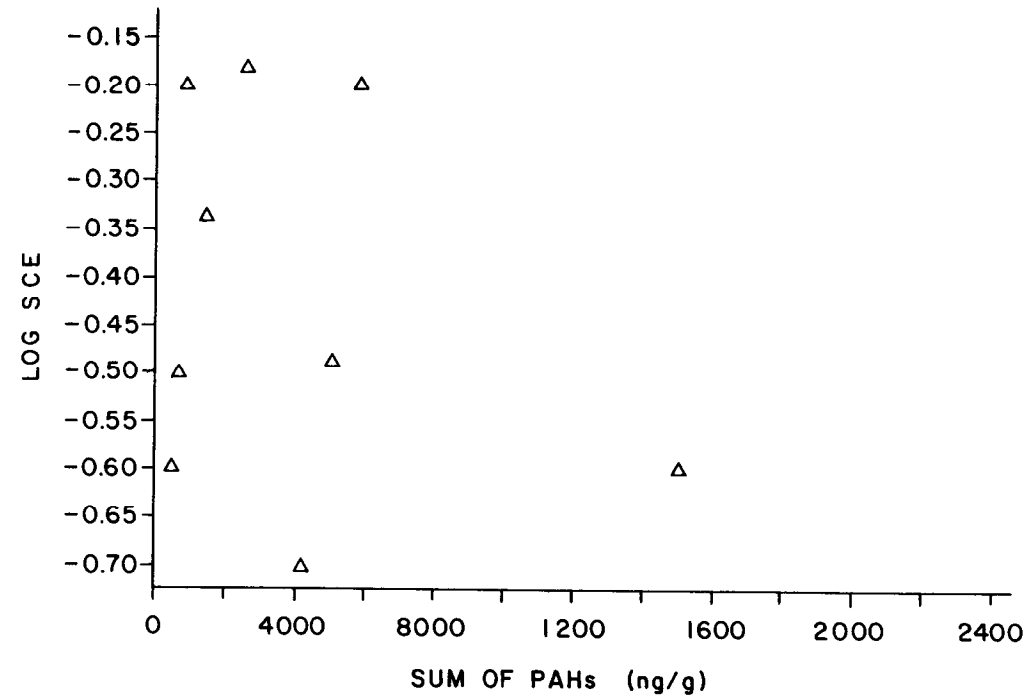


a. Fluoranthene

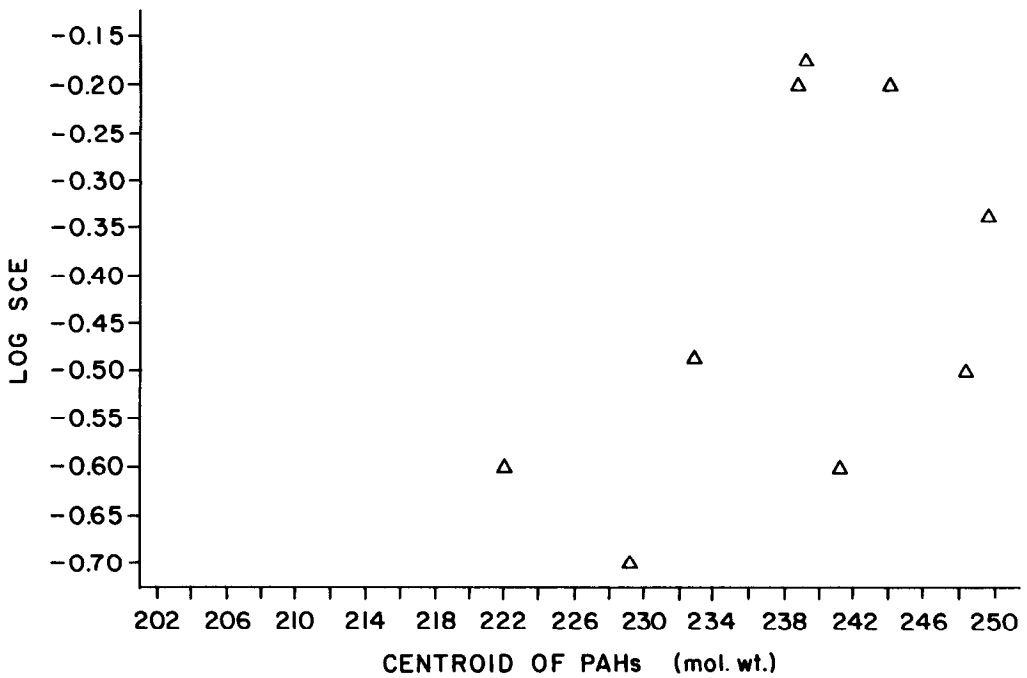


b. Benzo(a)pyrene

Figure 24. Relationship between SCE response and concentrations of fluoranthene and benzo(a)pyrene measured in tissues of field-collected *N. incisa*

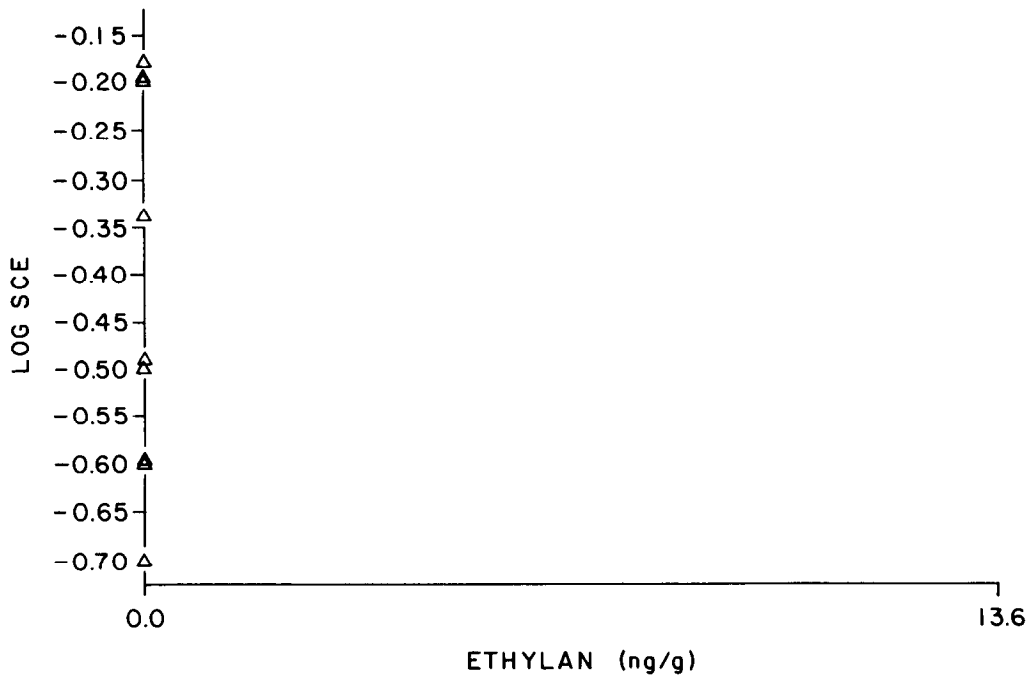


a. SUM of PAHs

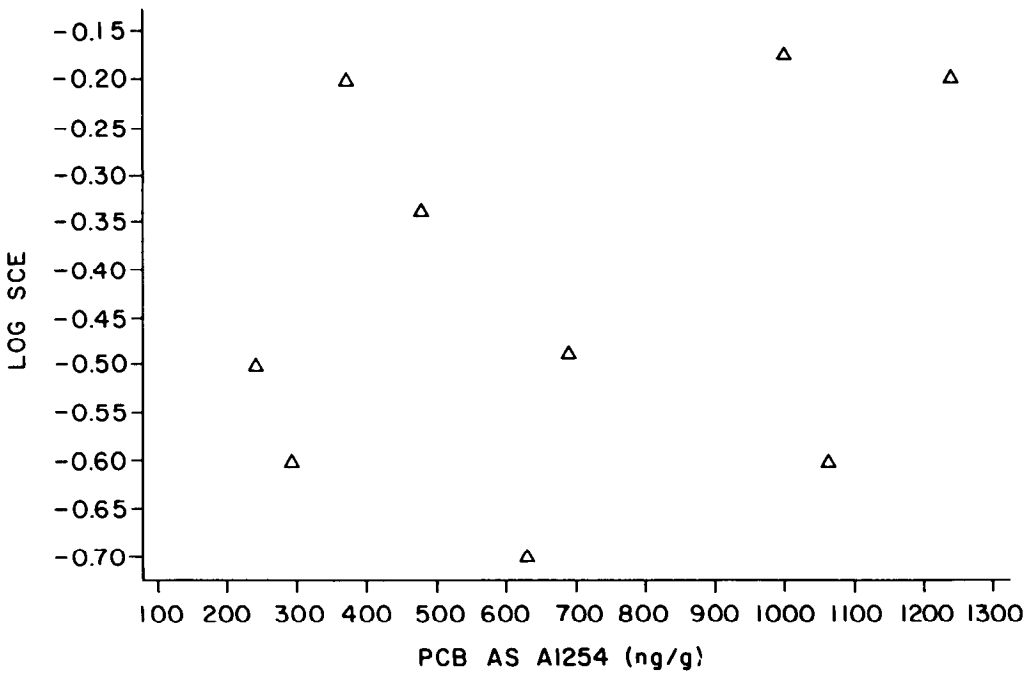


b. CENT of PAHs

Figure 25. Relationship between SCE response and concentrations of the SUM of PAHs and CENT of PAHs measured in tissues of field-collected *N. incisa*

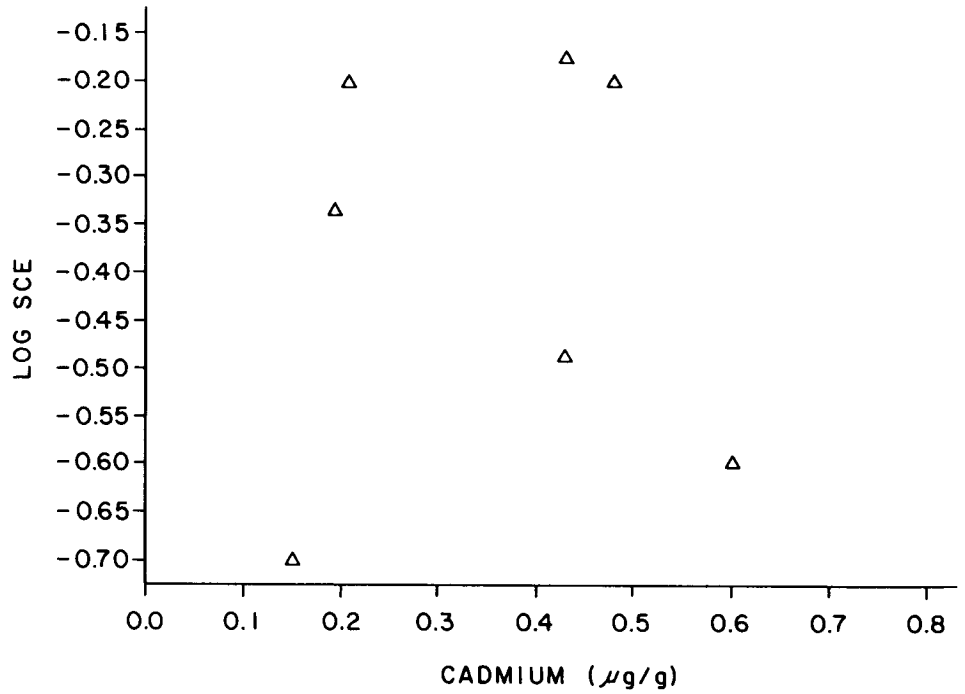


a. Ethylan

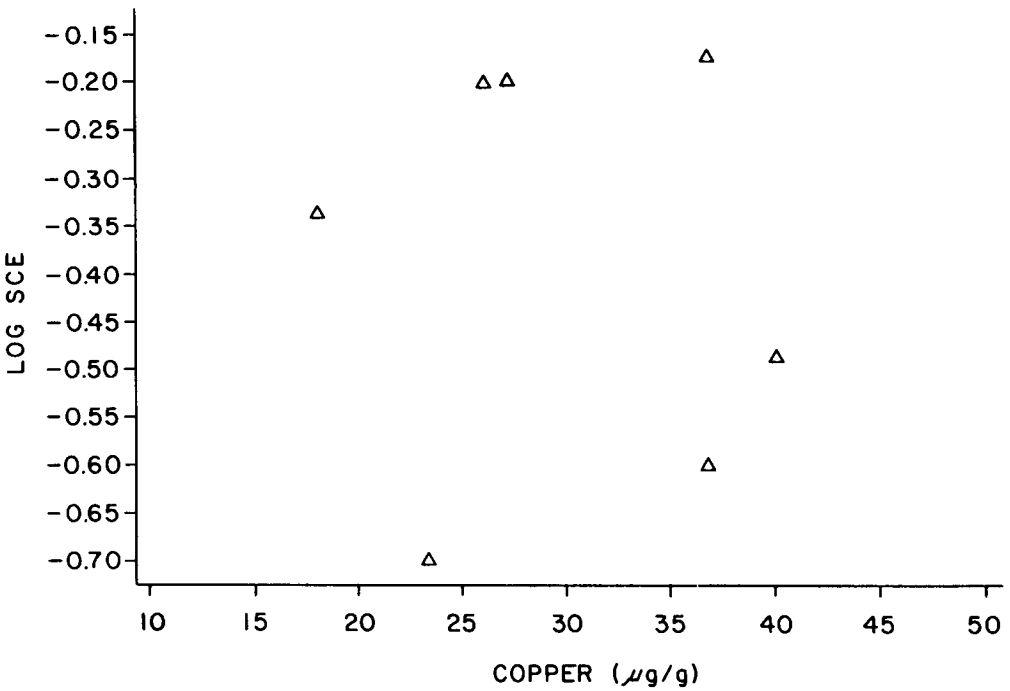


b. PCBs as A1254

Figure 26. Relationship between SCE response and concentrations of ethylan and PCBs as A1254 measured in tissues of field-collected *N. incisa*

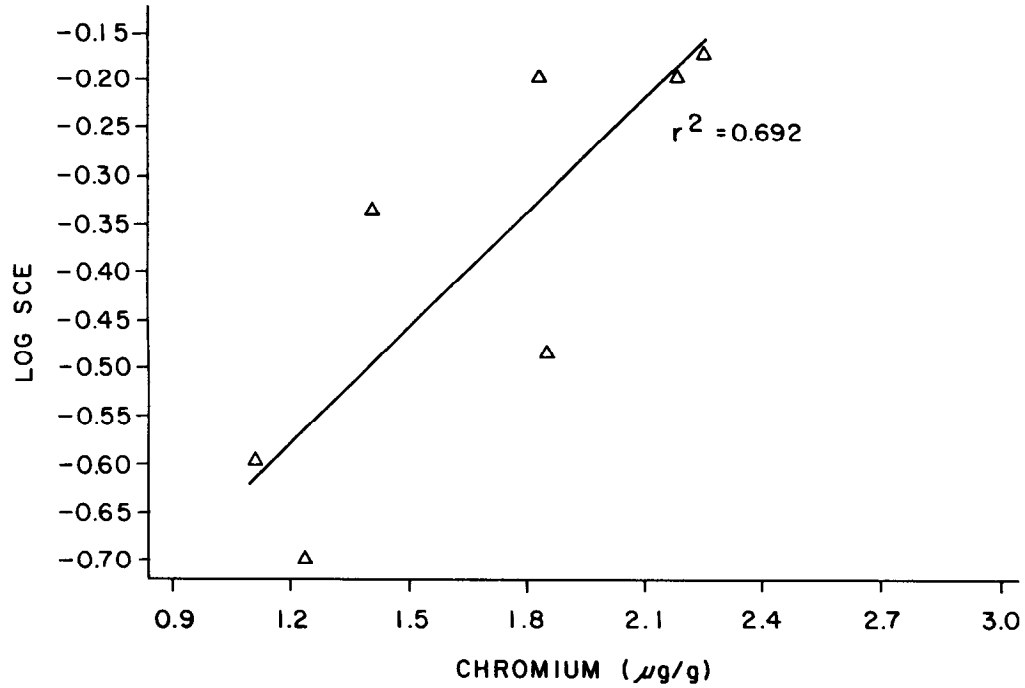


a. Cadmium

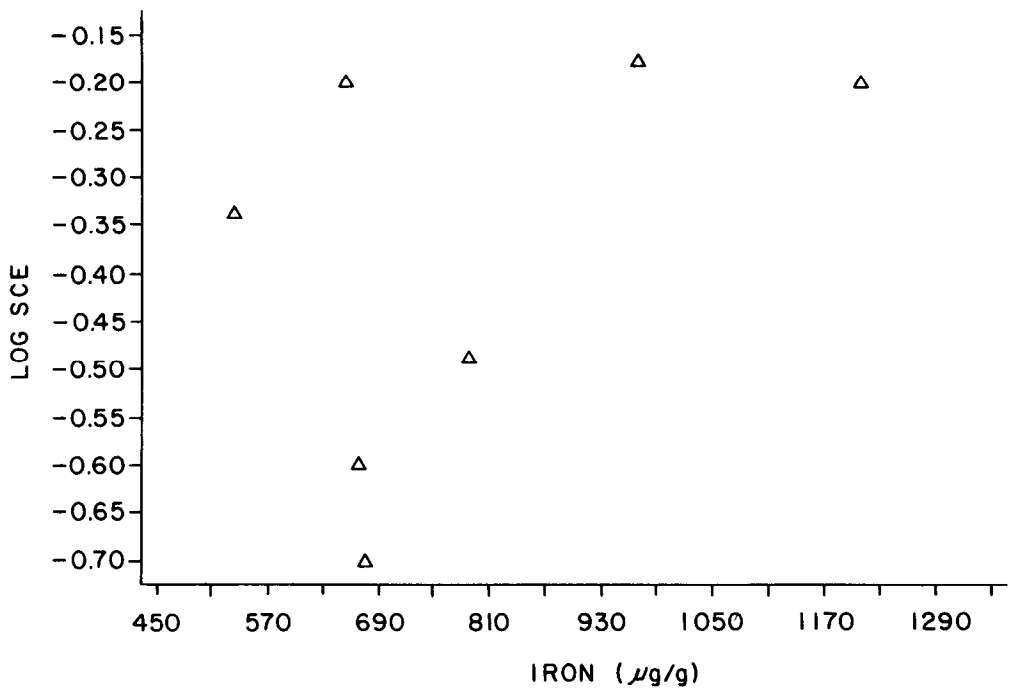


b. Copper

Figure 27. Relationship between SCE response and concentrations of cadmium and copper measured in tissues of field-collected *N. incisa*



a. Chromium



b. Iron

Figure 28. Relationship between SCE response and concentrations of chromium and iron measured in tissues of field-collected *N. incisa*

available for the regression analysis, seven for the inorganic and nine for the organic residues, make it improbable that relationships would be detected given the variability inherent in the data. With a sample size of seven, the correlation coefficient would have to exceed 0.75 ($r^2 = 0.56$) before it would be detected ($P \leq 0.05$) as significantly different from 0. There was a significant and direct relationship between SCE and chromium ($r^2 = 0.69$): as the chromium concentrations increased, the SCE response increased.

PART IV: DISCUSSION

98. The laboratory-field comparisons for effects and residues described in the following discussion should be viewed as qualitative for the following reasons: first, a quantitative relationship between the biological response (SCE) and an exposure concentration was not defined in the laboratory or in the field; and, second, field exposures were insufficiently described to permit defining relationships between SCE and exposure concentration in the CLIS environment. This is particularly important because the laboratory-field comparisons require accurate predictions of environmental exposures.

Laboratory Studies

99. SCE is a chromosomal response used to detect mutagens. Based on available literature (Latt et al. 1981), the utility of the SCE response has been evaluated in USEPA's Gene-Tox Program. It is extremely sensitive and can detect both direct-acting compounds and those that require metabolic activation. A positive SCE response generally indicates that a compound is mutagenic. The test gives few false positive results (Latt et al. 1981). The correlation of SCE with mutation, the diversity of chemical classes inducing SCE, and the utility of SCE for estimating genetic risk were considered. It was concluded that the SCE response was excellent for detecting compounds that form reaction products with DNA.

100. The Gene-Tox panel, composed of national experts, recommended criteria for negative/positive conclusions for the SCE response (Latt et al. 1981). A weakly positive SCE response was classified as representing a statistically significant increase in SCE over baseline. A strongly positive SCE response was classified as representing at least a twofold SCE increase. If these decision criteria are applied to the results of the laboratory BRH experiment, with replicates pooled, the overall result is a weakly positive SCE response in the B/R treatment. If replicate 3 (Table 5) is considered alone, the SCE response in the B/R treatment represents a twofold increase over the response in the R/R treatment. This represents a strongly positive SCE response. The inconsistency in SCE response among the laboratory replicate tests suggests that one or more factors important in SCE induction under laboratory conditions was not controlled. One limitation of the SCE response

cited in the Gene-Tox Report is its sensitivity to subtle variations in the test organism. Inadequate activation of promutagens or altered reactivity of mutagens before reaching the target tissue (worm chromosomes in this case) may give false negative responses (Latt et al. 1981). The activity of the mixed-function oxygenase (MFO) system in *N. incisa* would be an important variable in these experiments because of the high concentrations of promutagens, such as PAHs, in BRH sediment. The MFO system is induced by exposure to contaminants such as PAHs and PCBs. This induction takes several weeks in polychaetes (Fries and Lee 1984). Therefore, the laboratory experiments (10 days) were not long enough to induce MFO activity and elicit an SCE response. Preconditioning of worms and thus the induction of MFO activity before their use in the laboratory BRH experiment was the most likely uncontrolled variable in this study. Hence, the application of SCE to *N. incisa* needs further development before it can be recommended for routine use.

101. The increased frequency of SCE observed in *N. incisa* is consistent with responses of the Ames test to extracts of BRH sediment. Whole extracts of BRH sediment elicited a dose-response with *Salmonella* strain TA100 in the presence of S-9. This means that the mutation was a base-pair substitution and that the mutagens in BRH sediment needed to be metabolized before being reactive.* Based upon current understanding of mutagenesis, the positive results with the Ames test and the SCE response in *N. incisa* suggest a tumorogenic potential for BRH sediment. This is supported by Gardner et al. (1986), who report tumor development in American oysters and winter flounder exposed to BRH sediment under laboratory and field conditions.

102. The increased frequency of SCE observed in *N. incisa* is consistent with the chemical composition of BRH sediment to which the worms were exposed. This conclusion follows from the fact that many organic chemicals found in three standard solvent fractions of BRH sediment, and some selected inorganic compounds also identified in the sediment (Rogerson, Schimmel, and Hoffman 1985), have been reported to be DNA-damaging agents.

103. The BRH sediment was separated by solvent extraction into three fractions, PF-50, F-2, and F-3 (Rogerson, Schimmel, and Hoffman 1985). The PF-50 fraction is characterized as nonpolar and is a PCB fraction. For BRH

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* T. C. Lee and A. Senecal. Unpublished data generated under a USEPA Cooperative Agreement with the University of Rhode Island, #CR-812807-01-0.

sediment, this fraction contained a variety of PCBs, saturated aliphatic hydrocarbons and cycloalkanes, dichlorodiphenyl-dichloroethylene (DDE), and some two-ring aromatic hydrocarbons (acenaphthene, acenaphthylene, biphenyl, and naphthalene). The weight of the evidence suggests that, in general, PCBs are not genotoxic. Despite early reports that some PCBs may bind to DNA (Allen and Norback 1975; Wyndham, Devenish, and Safe 1976) and are mutagenic to *Salmonella typhimurium* (Wyndham, Devenish, and Safe 1976), more recent studies (Schoeny 1982; IARC 1978, 1982b) fail to support the earlier findings. PCBs may, however, contribute indirectly to the in vivo genotoxicity of promutagens by inducing MFO system enzymes important in metabolizing promutagens to active forms (Ramel 1975). There is no evidence that the aliphatic hydrocarbons or their cyclic counterparts, the cycloalkanes, are genotoxic; however, the paucity of the literature regarding the genotoxic properties of these compounds suggests that little testing has been done. DDE is reported in one study (Sina et al. 1983) to induce DNA single strand breaks in rat hepatocytes, and acenaphthylene is reported to induce mutation in *S. typhimurium* (Kaden, Hites, and Thilly 1979). Many other studies, however, report negative results for these and other PF-50 fraction compounds (McCann et al. 1975; Moriya et al. 1983; Mitchell et al. 1983; Mamber, Bryson, and Katz 1983, 1984; Zimmermann et al. 1984).

104. The F-2 fraction is a slightly polar aromatic hydrocarbon fraction. For BRH sediment, this fraction contained many PAHs (fluorene, phenanthrene, dibenzothiophene, fluoranthene, pyrene, chrysene, benzanthracene, benzo(a)pyrene, benzo(e)pyrene, perylene, and various alkylated deriatives of these compounds, as well as benzoperylene, dibenzanthracene, coronene, and dibenzochrysene. Dichlorodiphenyltrichloroethane (DDT), chlordanes (hepta, octa, and nonachlor), and ethylan were also found in this fraction. Several of these compounds have been reported to be genotoxic in a number of short-term assays, usually following metabolic activation (IARC 1983). Genotoxic effects have been reported for ten F-2 fraction compounds (fluorene, pyrene, benzanthrancene, chrysene, benzo(a)pyrene, benzo(e)pyrene, perylene, benzoperylene, bidenzanthracene, and coronene), although the evidence for three of these chemicals (perylene, benzoperylene, and coronene) is not sufficient to justify a genotoxic classification (IARC 1983). There is, however, limited or sufficient evidence that pyrene, benzanthracene, chrysene, benzo(a)pyrene, benzo(e)pyrene, and dibenzanthracene induce SCE in several in vitro or in vivo

assay systems (IARC 1983). There is limited evidence that chlordane is genotoxic (IARC 1979) and substantial evidence that DDT is not genotoxic (IARC 1982b).

105. The F-3 fraction is more polar than the F-2 fraction and typically contains ketones, quinones, carbazoles, and phthalates. These chemical classes were identified in the F-3 fraction of BRH sediment. Evidence for the genotoxicity of these chemicals is limited. Natural quinones form a large class of colored pigments, occurring mainly in higher plants and some microorganisms, particularly the lower fungi. Several natural anthraquinones have been reported to be strongly mutagenic in *Salmonella* (strain TA2637) with metabolic activation (Tikkanen, Matsushima, and Natori 1983), possibly via the generation of active oxygen (Brown 1980; Chesis et al. 1984; Lesko and Lorentzen 1985; Smith 1985). In addition, several substituted anthraquinones have recently been reported to induce chromosomal aberrations and SCE in cultured mammalian cells (Nishio et al. 1982). Some substituted carbazoles have been evaluated for genotoxic effects, primarily in procaryotic organisms, and reported to be genotoxic (IARC 1983). The genotoxicity of phthalates has been studied extensively. Generally, phthalates have not been genotoxic in several diverse test systems (IARC 1982a; Butterworth et al. 1984).

106. Potential contributions from inorganic compounds to the genetic effects observed in *N. incisa* must also be considered. From the genetic viewpoint, the most important inorganic elements identified in BRH sediment were arsenic, cadmium, chromium, copper, lead, manganese, and nickel. Concentrations of cadmium, chromium, and copper were particularly high, exceeding reference sediment concentrations by factors of 100, 30, and 46, respectively (Rogerson, Schimmel, and Hoffman 1985). Although only hexavalent chromium has been reported to consistently induce gene mutations in procaryotic test systems (Rossman 1981), other inorganic compounds have been reported to induce a variety of chromosomal effects, including SCE. Arsenic, cadmium, chromium, lead, nickel, and zinc have been reported to induce chromosomal aberrations in animals, plants, and cultured cells (IARC 1980; Bianchi et al. 1983; Garrett et al. 1984; Ma et al. 1984; Ochi et al. 1984), whereas arsenic, chromium, and nickel have also been reported to induce SCE (Bianchi et al. 1983; Garrett et al. 1984; Newman, Summitt, and Nunez 1982). In addition, arsenic, copper, and manganese have recently been reported (Rossman and Molina 1986) to be comutagens by enhancing the mutagenic activity of known mutagenic agents.

107. In summary, although it is difficult to link any of the chemicals found in the PF-50 fraction to the genetic lesions observed in *N. incisa*, the presence of (a) many PAHs in F-2, (b) possible substituted anthraquinones and carbazoles in F-3, and (c) several inorganic substances, all capable of inducing SCE, predicts that this response will occur in organisms exposed to BRH sediment.

Field Studies

108. There are two clear patterns in the SCE data from the field studies. First, the SCE response is low in June 1983, rises significantly in August 1983, then declines in December 1983. Second, there are no differences among stations on any given date. If the Gene-Tox decision criteria are applied, the June to August increase represents a strongly positive SCE response. This increase in SCE frequency is not a seasonal effect. The data in Figure 22 demonstrate this clearly. Worms for the laboratory test were collected at REFS during the cruise for the field observations. The laboratory control worms (R/R) exhibit an SCE frequency one half that of the field REFS site worms. The only difference between these two samples of worms was the length of time in the laboratory. Ambient temperature in CLIS at the time of collection was 20.8° C. The worms were held at 20° C in the laboratory. Salinities were comparable also.

109. The August field data (Figure 22) indicate an increased SCE frequency. The SCE frequency declines when the worms are held in clean laboratory conditions. The SCE frequency in the laboratory-held worms returns to field levels when the worms are exposed to BRH sediments. Thus, there is a strong, positive correlation between exposure to BRH sediments and SCE response.

110. To understand the SCE response in the field, the pattern of exposure to dredged material and the nature of the SCE response need to be examined. The SCE response at all FVP stations and REFS implies exposure throughout the FVP study site. There is evidence suggesting dispersion of dredged material during disposal.

111. The amount of BRH material disposed at the FVP site constituted about 5 percent of the total volume of dredged material disposed in CLIS during the spring of 1983 (Figure 29). The most active site in CLIS was the MQR

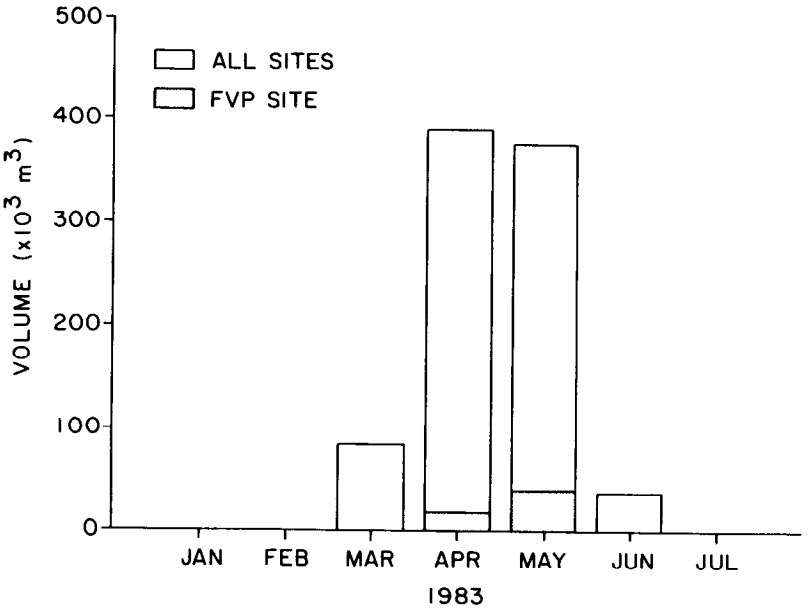

Figure 29. Volumes of dredged material disposed at all sites within the CLIS disposal area during the spring of 1983

site which received more than 500,000 cu m of dredged material (Morton et al. 1984). This site is located approximately 3 km from the FVP study site and 2 km from REFS. Disposal at the MQR site began 9 March and, therefore, was ongoing during the *Mytilus edulis* monitoring program at FVP sites which began on 16 March 1983. The *M. edulis* from this monitoring program had elevated tissue contaminant concentrations at FVP stations before disposal began at the FVP site. For example, the *M. edulis* from CNTR sampled prior to disposal at the FVP site had elevated concentrations of phenenathrene, fluoranthene, sum of the alkyl homologs, and SUM of PAHs (Nelson et al. 1987).

112. During disposal of BRH dredged material at the FVP site, the *M. edulis* from 1000E accumulated elevated tissue concentrations of phenanthrene, fluoranthene, sum of alkyl homologs, SUM of PAHs, benzo(a)pyrene, PCBs, and ethylan (Nelson et al. 1987). These same compounds were found at elevated concentrations in the surficial (0-2 cm) sediments at 1000E (Appendix A). *Nephtys incisa* sampled from the sediments at 1000E accumulated elevated tissue concentrations of phenanthrene, fluoranthene, sum of alkyl homologs, SUM of PAHs, benzo(a)pyrene, and PCBs.

113. The *M. edulis* tissue concentration data from 1000E indicate that, during disposal at the FVP site, the BRH material dispersed as far as 1000E. The sediment chemistry indicates that BRH material settled on the bottom at

1000E. The tissue concentration data for *N. incisa* indicate that the worms accumulated contaminants associated with the sediments at 1000E.

114. During the summer of 1983, tissue concentrations of PCBs in *N. incisa* increased at all FVP stations and reached their highest measured concentrations in September. Since there were no significant storms during the summer of 1983, the contaminant exposures were probably due to initial dispersion of dredged material, and tidally driven resuspension and movement of sediments from the dredged material mounds.

115. To understand why the SCE response in *N. incisa* had not been initiated by 3 weeks postdisposal and then why, when it was initiated, the response was the same throughout the entire CLIS study area, the biology of the SCE response needs to be examined.

116. SCE is a visual consequence of an effect directly on the DNA. If there is genotoxicity associated with BRH sediments, a likely cause is the PAHs. These compounds are not direct-acting mutagens. They need to be metabolized to reactive intermediates. A key enzyme system in this metabolism is the cytochrome P-450 dependent MFO. These enzymes are not functioning at all times. Their reactivity is induced, that is, greatly increased, by exposure to substrate compounds (PAHs). This induction is not immediate. Fries and Lee (1984) report an induction time of 4 to 8 weeks for the MFO system in the marine polychaete *Nereis virens* exposed to benzo(a)pyrene. The SCE observations were made on young-of-the-year *N. incisa*. *Nephtys incisa* spawns in CLIS during April and May (Carey 1962). The larvae settle from their planktonic stage on to the sediments during May and June (Zajac and Whitlatch 1985). Therefore, the SCE data from field worms collected 3 weeks postdisposal may well be from worms whose MFO system had not been activated; and, therefore, one would not expect to see an increase in the SCE response.

117. The tissue concentrations of PCBs and PAHs in *N. incisa* during the summer of 1983 indicate that the MFO system in the exposed worms had been activated by 14 weeks postdisposal. PCB tissue concentrations reached a peak in September, 4 months postdisposal, indicating a continuous exposure to contaminated sediments during this period. In contrast, the highest PAH tissue concentrations occurred in July, only 2 months postdisposal. These data suggest that metabolism of PAHs was induced and was causing a sharp decline in tissue PAH concentrations despite continuous exposure to these compounds.

Presumably, by 14 weeks postdisposal, the worm MFO system had been induced, resulting in a significant SCE response.

118. When the SCE response occurred, it occurred to the same extent at all four biological stations (Table 9). To understand this, one needs to examine the nature of SCE responses to PAHs and complex mixtures. In both cases, the usual response is a doubling of SCE over background and then no further increase. The biological explanation of this "plateau" is not clear, but it has been observed in widely different test systems. For benzo(a)pyrene this plateau has been observed in vitro in human cells (Juhl et al. 1978; Abe 1984; Hopkin 1984), in Chinese hamster cells (Pal et al. 1978; Abe 1984; Bloom 1984; Hopkin 1984), and in rat cells (Abe 1984). The plateau effect has been observed in in vivo studies involving rabbits (Takehisa and Wolff 1978), in a marine fish (Stromberg, Landolt, and Kocan 1981), and in a polychaete (Pesch, Pesch, and Malcolm 1981). In two separate studies involving exposure of a freshwater fish, *Umbra pygmae*, to a complex mixture (polluted Rhine River water), a twofold to threefold increase in SCE was found (Hooftman and Vink 1981; Alink et al. 1980).

119. In the present study, SCE frequencies in worms from the REFS site increased by a factor of 3.8 (Table 9) between 6 June (T + 3 weeks) and 26 August (T + 14 weeks). The SCE frequencies in worms from the other three FVP stations increased to the same extent during this period, even though tissue residue analyses indicate they had been exposed to much higher BRH concentrations. The simplest explanation for this response pattern is that the plateau had been reached at the REFS station; therefore, the SCE response was insensitive to any further increase at the other three stations, despite higher exposure concentrations of BRH. The laboratory experiment supports the hypothesis of a plateau. The greatest increase in SCE frequency observed in the laboratory (treatment B/R in Replicate 3, Table 5), at exposures higher than estimated for the field, was statistically the same as the 26 August field data. An SCE frequency of about 0.6 SCE/chromosome appears to be the plateau for *N. incisa* exposed to BRH sediments, and by inference the exposure levels needed to reach this plateau were present at all of the FVP stations during the summer of 1983.

Laboratory-to-Field Comparisons

120. A primary objective of this program was to field verify the laboratory biological responses by measuring the same response under both laboratory and field exposures. A basic and often implicit assumption is that results derived from laboratory tests are directly applicable in the field. This study is designed to test this assumption.

121. Exposure conditions must be examined to determine whether the biological responses are responding to comparable situations in the laboratory and the field. Physical data were used to make three different estimates of exposure to BRH material at the FVP stations. Water chemistry data were used to estimate milligrams of BRH per litre 1 m above the bottom at the FVP stations (Nelson et al. 1987). With the assumption of a 10× enrichment from the 1 m above the bottom value, there is a predicted exposure at the sediment-water interface of 6 to 13 mg BRH/ℓ at the FVP stations as a result of disposal at the FVP site. Estimates of exposure via resuspension of surficial sediments predict much higher concentrations. A worst case estimate assumes that all of the predicted suspended solids are BRH material from the disposal mound. This estimate predicts up to 100 mg BRH/ℓ under quiescent conditions and up to 300 mg BRH/ℓ under storm conditions. A more probable estimate assumes that sediments resuspended at each station are the source of contaminants for the suspended solids. This estimate predicts a graded exposure at the FVP stations with maximum values of 40 mg/ℓ at 200E, 12 mg/ℓ at 400E, and 4 mg/ℓ at 1000E for quiescent conditions. These values increase to 120 mg/ℓ at 200E, 40 mg/ℓ at 400E, and 10 mg/ℓ at 1000E for storm conditions.

122. If it is assumed that tissue concentrations in *N. incisa* are directly related to exposure concentrations, this relationship may be used to test the reasonableness of the exposure predictions. This assumption is reasonable, based on results from laboratory experiments. A cluster analysis of all *N. incisa* tissue residue data revealed no consistent clustering of the laboratory data separate from the field data. Any apparent clusters included both laboratory and field data. Therefore, if it is assumed that tissue concentrations reflect exposure concentrations, then this association of laboratory and field tissue concentration data indicates an overlap of laboratory exposure conditions with field exposure conditions. The estimates of field exposures to BRH sediment (mg/ℓ) suspended at the sediment-water interface

based on PCB concentrations in field-collected *N. incisa* are up to 12 mg/ℓ at REFS, 88 mg/ℓ at 1000E, and 130 mg/ℓ at 400E.

123. Assuming that exposures were due to initial dispersion of BRH sediments during disposal and subsequent resuspension and movement of sediments from the dredged material mound, a combination of estimates seems appropriate. The estimate based on water chemistry predicts exposures of at least 6 mg/ℓ at the sediment-water interface at all FVP stations during disposal activities in CLIS. The worst case resuspension estimate predicts exposures of up to 100 mg/ℓ in the vicinity of the disposal mound. These estimates (6 to 100 mg/ℓ) agree well with those predicted by the tissue concentration exposure concentration relationship (12 to 130 mg/ℓ). The laboratory exposures for the SCE response were 0 and 200 mg BRH/ℓ as suspended solids. These exposures overlap the estimated range of exposures in the field, simulated clean control conditions at REFS, and worst case storm conditions near the disposal mound.

124. The SCE response in field worms ranged from a station mean (SCE/chromosome/station) of 0.08 to 0.66. The means for all stations for the three sampling dates were 0.16 in June, 0.60 in August, and 0.30 in December. The SCE response in laboratory worms ranged from a treatment mean (SCE/chromosome/treatment) of 0.20 to 0.63. The overall means for the four laboratory treatments in the three replicates ranged from 0.27 to 0.42. The range of SCE/chromosome/sample response in the laboratory (0.20 to 0.63) agreed well with the range in the field (0.08 to 0.66).

125. When an SCE response to contaminated sediment was elicited in *N. incisa*, the response was comparable in both the laboratory and the field. This is illustrated best with data from *N. incisa* collected on 26 August 1983. The worms for both the laboratory and the field observations were collected at the same time, on the same cruise. These data (Figure 23) demonstrate that, under certain conditions (B/R), the laboratory worms responded in a manner directly comparable with those exposed in the field.

Residue-Effects Comparisons

126. Toxicology is a multidisciplinary science concerned with describing and explaining the adverse effects of chemicals and other agents on living organisms. Genetic damage is a special type of adverse effect. Genotoxic agents differ from other kinds of toxins in that (a) they damage the genetic

material of organisms; (b) this damage, if not lethal, is generally irreversible and may show delayed expression; and (c) effects are additive and accumulate with time. Not all chemicals are capable of causing such damage. Because SCE correlates with mutagenic activity, residue-effect relationships between SCE and tissue concentration of contaminants would be expected for only a limited subset of the compounds identified in BRH material. Of 67 compounds identified in BRH material, 13 (19 percent) are known mutagens. The list of mutagens includes 7 of 57 (12 percent) organic compounds and homologs and 6 of 10 (60 percent) inorganic compounds.

127. Based upon this, one might expect a direct correlation between tissue concentration of known mutagens and the SCE response. This is not the case, particularly for the seven organic compounds, since all seven are PAHs. It is not the PAH compounds themselves that are genotoxic. Genotoxic action is associated with their metabolic products. Therefore, a simple correlation between tissue concentration of parent PAH compounds and genetic response will not generally exist.

128. Of the ten chemicals analyzed for correlation between SCE response, only two, benzo(a)pyrene and chromium (in the hexavalent state), are known mutagens. Benzo(a)pyrene is a PAH and therefore is not a direct-acting mutagen. Its metabolic products are genotoxic (Bresnick 1976). Hexavalent chromium is a direct-acting mutagen (Rossman 1981). Chromium is, therefore, the only one of the twelve chemical variables for which a relationship could be expected between tissue concentration and SCE. It is satisfying that the expected was found. There seems to be a direct relationship between chromium concentrations and SCE response. The relationship between sediment concentrations of chromium and SCE response in exposed organisms deserves further research. However, the FVP study was not designed to address cause-effect relationships. The multicontaminant nature of the dredged material precludes any such assumptions.

PART V: CONCLUSIONS

129. There were three primary objectives in the FVP. The first objective was to test the applicability of the SCE technique to measure mutagenic effects of dredged material and to determine the degree of variability and reproducibility inherent in the procedure. The second objective was to field verify the response observed in the laboratory and determine the accuracy of the laboratory prediction. The third objective was to determine the degree of correlation between tissue residues accumulated from dredged material and the response in SCE.

130. To address the first objective, the SCE technique was applied to *N. incisa*, an infaunal polychaete dominant in the benthic community at the CLIS disposal site. The laboratory phase was replicated three times with *N. incisa* using a randomized complete block design. When the data from all three replicate tests were pooled and analyzed statistically, the frequency of SCE response in the B/R treatment was significantly higher than all other treatments. The inconsistency in SCE response among the laboratory experiments suggests that one or more factors important in SCE induction under laboratory conditions were not controlled. Preconditioning of worms and thus the induction of MFO activity before their use in the laboratory BRH experiment was the most likely uncontrolled variable in this study. Clearly, the application of SCE to *N. incisa* needs further development before it can be recommended for routine use.

131. For comparison with laboratory data (the second objective of the FVP), field data are available for three sampling dates: 6 June 1983 (T + 3 weeks), 26 August 1983 (T + 14 weeks), and 13 December 1983 (T + 28 weeks). The August field data indicate an increased SCE frequency. The SCE frequency declines when the worms are held in clean laboratory conditions. The SCE frequency in the laboratory-held worms returns to field levels when the worms are exposed to BRH sediments. Thus, there is a direct and positive correlation between frequency of SCE response and exposure to BRH sediments. Therefore, the laboratory and field data agreed well.

132. Finally, the third objective was addressed by correlating tissue concentrations of ten chemicals with SCE response. Only two of these chemicals, benzo(a)pyrene and chromium, are known mutagens. Benzo(a)pyrene is not a direct study mutagen; therefore, a correlation with SCE was not expected and

did not occur. Chromium is a direct-acting mutagen, and a significant correlation was found. The relationship between sediment concentrations of chromium and SCE response in exposed organisms deserves further research. However, the FVP study was not designed to address cause-effect relationships. The multicontaminant nature of the dredged material precludes any such assumptions.

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APPENDIX A: CHEMICAL ANALYSIS OF SURFICIAL SEDIMENTS

Table A1

Phenanthrene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	114
12/08/82	--	--	--	--	77
03/02/83	105	101	132	--	107
03/02/83	--	--	--	--	98
03/02/83	--	--	--	--	62
06/03/83	1,560	1,960	910	52	88
06/03/83	--	--	--	63	--
07/26/83	770	1,710	240	174	51
09/01/83	780	1,010	220	168	94
09/01/83	--	--	--	--	81
03/19/84	77	98	100	250	42
03/20/84	--	--	141	78	90
03/20/84	--	--	--	--	76
03/20/84	200	--	--	--	--
09/11/84	147	57	116	109	40
10/16/84	230	--	85	137	123
10/22/85	43	440	38	69	51

Table A2

178 Alkyl Homolog Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	210
12/08/82	--	--	--	--	172
03/02/83	250	210	260	--	188
03/02/83	--	--	--	--	230
03/02/83	--	--	--	--	127
06/03/83	--	--	5,300	230	189
06/03/83	--	--	--	122	--
07/26/83	9,700	--	1,500	412	131
09/01/83	5,200	--	1,480	613	186
09/01/83	--	--	--	--	189
03/19/84	1,330	590	560	600	103
03/20/84	--	--	590	260	170
03/20/84	--	--	--	--	185
03/20/84	1,200	--	--	--	--
09/11/84	3,000	270	640	250	103
10/16/84	1,260	--	240	420	240
10/22/85	490	3,800	430	210	192

Table A3

Fluoranthene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	280
12/08/82	--	--	--	--	200
03/02/83	300	260	340	--	270
03/02/83	--	--	--	--	230
03/02/83	--	--	--	--	148
06/03/83	2,300	2,300	1,240	142	220
06/03/83	--	--	--	161	--
07/26/83	1,940	2,600	570	400	140
09/01/83	1,370	2,800	560	380	220
09/01/83	--	--	--	--	210
03/19/84	290	330	330	600	124
03/20/84	--	--	360	210	230
03/20/84	--	--	--	--	185
03/20/84	510	--	--	--	--
09/11/84	650	166	410	250	108
10/16/84	580	--	240	320	300
10/22/85	172	1,770	142	196	189

Table A4

Benzo(a)pyrene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	280
12/08/82	--	--	--	--	220
03/02/83	260	270	310	--	220
03/02/83	--	--	--	--	210
03/02/83	--	--	--	--	173
06/03/83	1,640	1,490	810	122	210
06/03/83	--	--	--	158	--
07/26/83	1,520	1,750	380	370	169
09/01/83	1,000	2,100	570	320	200
09/01/83	--	--	--	--	230
03/19/84	220	350	260	450	155
03/20/84	--	--	400	280	240
03/20/84	--	--	--	--	185
03/20/84	460	--	--	--	--
09/11/84	600	230	400	260	111
10/16/84	450	--	240	320	290
10/22/85	280	1130	230	196	380

Table A5

SUM PAH* Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	5,200
12/08/82	--	--	--	--	4,500
03/02/83	5,100	4,900	5,900	--	4,400
03/02/83	--	--	--	--	4,300
03/02/83	--	--	--	--	3,300
06/03/83	62,000	59,000	30,000	2,400	3,900
06/03/83	--	--	--	3,000	--
07/26/83	54,000	63,000	10,100	7,200	3,200
09/01/83	33,000	71,000	13,500	7,200	3,600
09/01/83	--	--	--	--	4,300
03/19/84	7,200	7,100	6,200	9,300	2,700
03/20/84	--	--	7,300	4,500	3,600
03/20/84	--	--	--	--	4,300
03/20/84	11,100	--	--	--	--
09/11/84	18,600	4,400	8,600	5,000	2,000
10/16/84	11,500	--	4,800	6,700	5,800
10/22/85	5,400	34,000	4,900	3,800	5,400

* PAH = polynuclear aromatic hydrocarbons.

Table A6

Centroid Statistic in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	249.7
12/08/82	--	--	--	--	252.0
03/02/83	247.6	248.9	247.7	--	247.4
03/02/83	--	--	--	--	248.0
03/02/83	--	--	--	--	252.1
06/03/83	238.7	234.1	235.2	241.4	248.3
06/03/83	--	--	--	250.3	--
07/26/83	234.7	232.6	234.4	247.3	252.5
09/01/83	239.7	238.6	244.7	244.3	245.4
09/01/83	--	--	--	--	250.3
03/19/84	237.0	245.1	241.1	244.5	251.0
03/20/84	--	--	243.5	245.3	243.7
03/20/84	--	--	--	--	251.5
03/20/84	242.9	--	--	--	--
09/11/84	240.8	249.2	244.1	247.5	247.2
10/16/84	240.4	--	248.4	247.7	250.0
10/22/85	248.8	241.1	248.6	248.7	253.4

Table A7

Ethylan Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/08/82	--	--	--	--	0.0
12/08/82	--	--	--	--	0.0
03/02/83	0.0	0.0	0.0	--	0.0
03/02/83	--	--	--	--	0.0
03/02/83	--	--	--	--	0.0
06/03/83	340.0	370.0	163.0	5.0	0.0
06/03/83	--	--	--	0.0	--
07/26/83	0.0	950.0	90.0	35.0	0.0
09/01/83	210.0	670.0	30.0	15.0	0.0
09/01/83	--	--	--	--	0.0
03/19/84	74.0	50.0	36.0	31.0	0.0
03/20/84	--	--	12.0	0.0	0.0
03/20/84	--	--	--	--	0.0
03/20/84	23.0	--	--	--	--
09/11/84	96.0	14.0	64.0	3.0	0.0
10/16/84	12.0	--	2.0	7.0	0.0
10/22/85	8.0	820.0	4.0	5.0	0.0

Table A8

PCB* (A1254) Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
08/18/82	--	--	73	--	59
11/11/82	--	--	30	--	26
12/08/82	--	--	--	--	48
03/02/83	77	75	98	--	65
03/02/83	--	--	--	--	67
03/02/83	--	--	--	--	60
06/03/83	1,730	1,650	890	79	59
06/03/83	--	--	--	45	--
07/26/83	180	1,830	240	117	28
09/01/83	1,190	2,200	340	200	59
03/19/84	270	250	162	96	26
03/20/84	181	--	--	--	--
09/11/84	440	113	183	66	27
10/16/84	181	--	84	162	77
10/22/85	72	1,550	37	48	29

* PCB = polychlorinated biphenyls.

Table A9

Cadmium Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
03/04/83	0.36	0.34	1.06	0.29	0.24
03/04/83	0.39	0.35	0.44	0.21	0.22
03/04/83	0.35	0.49	0.32	0.25	0.22
06/03/83	17.00	13.90	7.30	0.74	0.22
06/03/83	12.40	14.70	4.20	0.58	0.21
06/03/83	13.00	12.90	3.70	0.64	0.19
07/26/83	5.40	11.70	1.14	0.64	0.22
09/01/83	4.10	9.80	0.84	0.68	0.18
09/01/83	21.00	8.70	3.60	0.76	--
12/09/83	8.80	8.70	3.30	1.02	--
03/19/84	2.10	1.11	0.85	1.80	0.20
03/19/84	--	0.87	--	--	--
03/19/84	--	0.23	--	--	--
06/12/84	3.10	4.80	0.37	0.39	--
09/11/84	3.70	0.73	0.97	0.30	0.20
12/20/84	9.30	2.50	0.32	0.72	--
10/22/85	0.45	8.30	0.29	0.32	0.16

Table A10

Chromium Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
03/04/83	56	39	59	59	48
03/04/83	53	57	43	58	52
03/04/83	45	56	56	60	54
06/03/83	870	680	340	69	49
06/03/83	780	740	191	72	48
06/03/83	800	600	155	74	48
07/26/83	120	519	69	66	44
09/01/83	310	600	106	79	56
09/01/83	680	380	160	79	--
12/09/83	520	660	117	126	--
03/19/84	100	52	54	86	47
03/19/84	--	140	--	--	--
03/19/84	--	40	--	--	--
06/12/84	138	210	41	52	--
09/11/84	153	41	128	55	44
12/20/84	550	175	47	88	--
10/22/85	54	430	57	59	40

Table A11

Copper Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
03/04/83	67	57	67	70	55
03/04/83	62	69	63	68	57
03/04/83	63	67	64	69	58
06/03/83	1,640	1,380	680	99	48
06/03/83	1,300	1,420	360	102	51
06/03/83	1,330	1,240	303	106	56
07/26/83	450	1,230	185	106	49
09/01/83	560	1,070	134	103	47
09/01/83	1,890	910	510	122	--
12/09/83	910	950	370	177	--
03/19/84	200	111	143	123	53
03/19/84	--	107	--	--	--
03/19/84	--	114	--	--	--
06/12/84	350	530	89	83	--
09/11/84	430	86	156	73	48
12/20/84	1,000	500	52	131	--
10/22/85	92	910	75	72	46

Table A12

Iron Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
03/04/83	21,000	17,100	22,000	23,000	19,700
03/04/83	20,000	22,000	18,900	23,000	21,000
03/04/83	18,400	21,000	21,000	23,000	22,000
06/03/83	17,100	19,200	23,000	21,000	21,000
06/03/83	19,300	19,000	22,000	21,000	19,000
06/03/83	17,900	18,700	23,000	22,000	21,000
07/26/83	15,200	16,700	21,000	16,800	21,000
09/01/83	15,100	19,300	21,000	18,400	19,700
09/01/83	26,000	15,100	--	16,400	--
12/09/83	16,500	21,000	19,600	17,500	--
03/19/84	5,800	17,300	20,000	18,700	21,000
03/19/84	--	16,600	--	--	--
03/19/84	--	15,600	--	--	--
06/12/84	6,500	17,100	19,800	15,600	--
09/11/84	12,600	17,400	18,400	18,200	21,000
12/20/84	18,100	17,300	17,400	18,000	--
10/22/85	9,900	17,200	18,100	18,900	17,000

Table A13

Percentage of Black Rock Harbor (BRH) Sediment in the Surficial Sediments (0-2 cm) and the Contaminants Used for the Percent Calculations

Date	Station			
	CNTR	200E	400E	1000E
	Percentage of BRH Sediment			
Jun 83	44.5	41.1	12.5	1.8
Jul 83	15.0	37.4	3.3	1.6
Sep 83	32.0	36.7	4.9	2.0
Dec 83	32.8	36.1	9.5	4.4
Mar 84	4.4	2.2	1.9	1.8
Jun 84	9.5	15.6	0.5	0.7
Sep 84	10.0	0.8	3.5	0.5
Oct 84	2.6	--	0.2	1.6
Dec 84	35.1	11.3	0.0	1.0
Oct 85	0.2	21.0	0.0	0.0

(Continued)

Table A13 (Concluded)

Date	Station			
	CNTR	200E	400E	1000E
		Contaminants Used		
Jun 83	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	Cd+Cu+Cr
Jul 83	PAH+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr
Sep 83	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr
Dec 83	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr
Mar 84	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cu+Cr
Jun 84	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr	Cu+Cr
Sep 84	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cu+Cr	PAH+PCB+Cu+Cr
Oct 84	PAH+PCB	--	PAH+PCB	PAH+PCB
Dec 84	Cd+Cu+Cr	Cd+Cu+Cr	Cu+Cr	Cu+Cr
Oct 85	PCB	PAH+PCB	PCB	PAH+PCB

APPENDIX B: CHEMICAL FORMULAS AND FIELD WORM RESIDUE CONCENTRATIONS

Table B1

Chemical Contaminants Selected for Measurement

in Both Field and Laboratory Studies

Chlorinated hydrocarbon pesticides

Polychlorinated biphenyls
Ethylan

Aromatic hydrocarbons ≥ molecular weight 166:

Compound Class	Molecular Weight
Fluorene	166
C-1* Fluorene	180
C-2* Fluorene	194
C-3* Fluorene	208
C-4* Fluorene	222
Phenanthrene	178
Anthracene	178
C-1* Phenanthrene/anthracene	192
C-2* Phenanthrene/anthracene	206
C-3* Phenanthrene/anthracene	220
C-4* Phenanthrene/anthracene	234
Fluoranthene	202
Pyrene	202
C-1* Fluoranthene/pyrene	216
C-2* Fluoranthene/pyrene	230
C-3* Fluoranthene/pyrene	244
C-4* Fluoranthene/pyrene	258
Benzanthracene/chrysene**	228
C-1* Benzanthracene/chrysene**	242
C-2* Benzanthracene/chrysene**	256
C-3* Benzanthracene/chrysene**	270
C-4* Benzanthracene/chrysene**	284

(Continued)

* C-1, C-2, C-3, and C-4 refer to the number of methyl groups substituted somewhere in the parent compound.

** These names are representative of the class of polynuclear aromatic hydrocarbons (PAHs) measured at each molecular weight.

Table B1 (Concluded)

Compound Class	Molecular Weight
Benzofluoranthenes	252
Benzo(e)pyrene	252
Benzo(a)pyrene	252
Perylene	252
C-1* Benzopyrene/perylene**	266
C-2* Benzopyrene/perylene**	280
C-3* Benzopyrene/perylene**	294
C-4* Benzopyrene/perylene**	308
Benzoperylene**	276
Dibenzanthracene**	278
Coronene	300
Dibenzocrysene**	302
Hetrocyclic aromatic compounds	
Dibenzothiopehen	184
C-1* Dibenzothiophene	198
C-2* Dibenzothiophene	212
C-3* Dibenzothiophene	226
C-4* Dibenzothiophene	240
Metals	
Cadmium	
Copper	
Chromium	
Iron	
Lead	
Manganese	
Nickel	
Zinc	

* C-1, C-2, C-3, and C-4 refer to the number of methyl groups substituted somewhere in the parent compound.

** These names are representative of the class of polynuclear aromatic hydrocarbons (PAHs) measured at each molecular weight.

Table B2

Complete Formulae for Calculating all SUM and CENT Variables

PSUM = POS166 + POS178 + POS202 + POS228 + POS252 + POS276 + POS278 + POS300 + POS302

HSUM = H1C166 + H2C166 + H3C166 + H4C166 + H1C178 + H2C178 + H3C178 + H4C178 + H1C202 + H2C202 + H3C202 + H4C202 + H1C228 + H2C228 + H3C228 + H4C228 + H1C252 + H2C252 + H3C252 + H4C252

SUM = POS166 + H1C166 + H2C166 + H3C166 + H4C166 + POS178 + H1C178 + H2C178 + H3C178 + H4C178 + POS202 + H1C202 + H2C202 + H3C202 + H4C202 + POS228 + H1C228 + H2C228 + H3C228 + H4C228 + POS252 + H1C252 + H2C252 + H3C252 + H4C252 + POS276 + POS278 + POS300 + POS302

PCENT = [POS166*166 + POS178*178 + POS202*202 + POS228*228 + POS252*252 + POS276*276 + POS278*278 + POS300*300 + POS302*302]/PSUM

HCENT = [H1C166*180 + H2C166*194 + H3C166*208 + H4C166*222 + H1C178*192 + H2C178*206 + H3C178*220 + H4C178*234 + H1C202*216 + H2C202*230 + H3C202*244 + H4C202*258 + H1C228*242 + H2C228*256 + H3C228*270 + H4C228*284 + H1C252*266 + H2C252*280 + H3C252*294 + H4C252*308]/HSUM

CENT = [POS166*166 + H1C166*180 + H2C166*194 + H3C166*208 + H4C166*222 + POS178*178 + H1C178*192 + H2C178*206 + H3C178*220 + H4C178*234 + POS202*202 + H1C202*216 + H2C202*230 + H3C202*244 + H4C202*258 + POS228*228 + H1C228*242 + H2C228*256 + H3C228*270 + H4C228*284 + POS252*252 + H1C252*266 + H2C252*280 + H3C252*294 + H4C252*308 + POS276*276 + POS278*278 + POS300*300 + POS302*302]/SUM

The sum of alkyl homologs of PAH molecular weight 178 (HOS178) is calculated according to the following formula:

HOS178 = H1C178 + H2C178 + H3C178 + H4C178

where

HOS178 = sum of C-1 to C-4 alkyl-substituted 178 PAHs

This statistic was chosen to describe the alkyl homologs because the 178 alkyl homologs are the most intense homologs within the Black Rock Harbor (BRH) PAH distribution and because they afford the greatest BRH to REFS concentration ratio. Alkyl homologs were included because of potential differences between them and parent PAHs.

Table B3

Tissue Residue Concentrations in *N. incisa* from the T - 39 Weeks Field Collection in CLIS (17 Aug 82)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--
Fluoranthene	--	--	--	--
Benzo(a)pyrene	--	--	--	--
Ethylan	--	--	--	--
PCB as A1254	--	189	--	210
SUM of PAHs	--	--	--	--
Centroid of PAHs	--	--	--	--
Copper	--	--	--	--
Cadmium	--	--	--	--
Chromium	--	--	--	--
Iron	--	--	--	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B4

Tissue Residue Concentrations in *N. incisa* from the T - 26 Weeks Field Collection in CLIS (16 Oct 82)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--
Fluoranthene	--	--	--	--
Benzo(a)pyrene	--	--	--	--
Ethylan	--	--	--	--
PCB as A1254	--	240	--	290
SUM of PAHs	--	--	--	--
Centroid of PAHs	--	--	--	--
Copper	--	--	--	--
Cadmium	--	--	--	--
Chromium	--	--	--	--
Iron	--	--	--	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B5

Tissue Residue Concentrations in *N. incisa* from the T - 13 Weeks Field Collection in CLIS (16 Feb 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	5.6	--	4.0
Sum of 178 alkyl homologs	--	67	--	34
Fluoranthene	--	37	--	26
Benzo(a)pyrene	--	19	--	10
Ethylan	--	0	--	0
PCB as A1254	--	340	--	290
SUM of PAHs	--	780	--	530
Centroid of PAHs	--	242.9	--	244.4
Copper	--	18.1	--	21
Cadmium	--	0.1	--	0.5
Chromium	--	1.3	--	1.9
Iron	--	570	--	770

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B6

Tissue Residue Concentrations in *N. incisa* from the T - 11 Weeks Field Collection in CLIS (04 Mar 83)*

Chemical Compound	Station				
	CNTR	200E	400E	1000E	REFS
Phenanthrene	--	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--	--
Fluoranthene	--	--	--	--	--
Benzo(a)pyrene	--	--	--	--	--
Ethylan	--	--	--	--	--
PCB as A1254	--	--	--	--	--
SUM of PAHs	--	--	--	--	--
Centroid of PAHs	--	--	--	--	--
Copper	36	39	37	42	26
Cadmium	0.8	0.5	0.7	1.0	0.6
Chromium	2.9	1.7	1.7	2.4	2.0
Iron	980	790	760	980	1,040

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B7

Tissue Residue Concentrations in *N. incisa* from the T - 5 Weeks Field Collection in CLIS (12 Apr 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	10.7	--	9.6
Sum of 178 alkyl homologs	0	79	0	50
Fluoranthene	--	47	--	35
Benzo(a)pyrene	--	24	--	21
Ethylan	--	0	--	0
PCB as A1254	--	390	--	340
SUM of PAHs	--	960	--	710
Centroid of PAHs	--	241.4	--	243.7
Copper	--	49	--	28
Cadmium	--	0.5	--	0.6
Chromium	--	3.9	--	2.1
Iron	--	1,360	--	930

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B8

Tissue Residue Concentrations in *N. incisa* from the T + 2 Weeks Field Collection in CLIS (02 Jun 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	360	60	6.2
Sum of 178 alkyl homologs	--	3,690	840	44
Fluoranthene	--	970	197	19
Benzo(a)pyrene	--	250	85	13
Ethylan	--	0	0	0
PCB as A1254	--	1,060	630	290
SUM of PAHs	--	15,100	4,200	420
Centroid of PAHs	--	221.9	229.3	241.0
Copper	--	37	23	--
Cadmium	--	0.6	0.2	--
Chromium	--	1.1	1.2	--
Iron	--	670	680	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B9

Tissue Residue Concentrations in *N. incisa* from the T + 7 Weeks Field Collection in CLIS (03 Jul 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	300	8.3	7.8
Sum of 178 alkyl homologs	--	3,700	260	79
Fluoranthene	--	650	49	31
Benzo(a)pyrene	--	420	66	19
Ethylan	--	0	0	0
PCB as A1254	--	1,160	630	290
SUM of PAHs	--	16,700	1,980	840
Centroid of PAHs	--	229.5	241.4	243.3
Copper	--	--	--	--
Cadmium	--	--	--	--
Chromium	--	--	--	--
Iron	--	--	--	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B10

Tissue Residue Concentrations in *N. incisa* from the T + 8 Weeks Field Collection in CLIS (13 Jul 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--
Fluoranthene	--	--	--	--
Benzo(a)pyrene	--	--	--	--
Ethylan	--	--	--	--
PCB as A1254	--	--	--	--
SUM of PAHs	--	--	--	--
Centroid of PAHs	--	--	--	--
Copper	--	27	37	--
Cadmium	--	0.3	0.5	--
Chromium	--	3.2	1.9	--
Iron	--	520	690	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B11

Tissue Residue Concentrations in *N. incisa* from the T + 16 Weeks Field Collection in CLIS (06 Sep 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	14.3	9.8	7.3
Sum of 178 alkyl homologs	--	890	420	66
Fluoranthene	--	165	111	37
Benzo(a)pyrene	--	195	85	27
Ethylan	--	0	0	0
PCB as A1254	--	1,240	1,000	370
SUM of PAHs	--	5,900	2,900	850
Centroid of PAHs	--	239.0	239.4	243.8
Copper	--	27	37	26
Cadmium	--	0.2	0.4	0.5
Chromium	--	1.8	2.3	2.2
Iron	--	650	970	1,210

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B12

Tissue Residue Concentrations in *N. incisa* from the T + 28 Weeks Field Collection in CLIS (29 Nov 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	48	5.8	3.4
Sum of 178 alkyl homologs	--	870	93	34
Fluoranthene	--	210	36	23
Benzo(a)pyrene	--	122	35	16
Ethylan	--	0	0	0
PCB as A1254	--	690	480	240
SUM of PAHs	--	5,100	1,330	550
Centroid of PAHs	--	232.7	249.4	248.4
Copper	--	40.0	17.8	--
Cadmium	--	0.4	0.2	--
Chromium	--	1.8	1.4	--
Iron	--	790	530	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B13

Tissue Residue Concentrations in *N. incisa* from the T + 44 Weeks Field Collection in CLIS (20 Mar 84)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	220	4.4	4.6	1.5
Sum of 178 alkyl homologs	1,100	950	18	1.2
Fluoranthene	270	230	31	24
Benzo(a)pyrene	159	132	22	10
Ethylan	0	0	0	0
PCB as A1254	650	580	350	220
SUM of PAHs	4,900	4,300	380	183
Centroid of PAHs	221.2	224.7	235.0	233.1
Copper	--	--	--	--
Cadmium	--	--	--	--
Chromium	--	--	--	--
Iron	--	--	--	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B14

Tissue Residue Concentrations in *N. incisa* from the T + 56 Weeks Field Collection in CLIS (13 Jun 84)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--
Fluoranthene	--	--	--	--
Benzo(a)pyrene	--	--	--	--
Ethylan	--	--	--	--
PCB as A1254	--	--	--	--
SUM of PAHs	--	--	--	--
Centroid of PAHs	--	--	--	--
Copper	174	--	44	39
Cadmium	1.0	--	0.6	0.7
Chromium	5.9	--	2.1	1.9
Iron	380	--	680	770

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B15

Tissue Residue Concentrations in *N. incisa* from the T + 73 Weeks Field Collection in CLIS (10 Oct 84)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	--	--	--	--
Sum of 178 alkyl homologs	--	--	--	--
Fluoranthene	--	--	--	--
Benzo(a)pyrene	--	--	--	--
Ethylan	--	--	--	--
PCB as A1254	--	--	--	--
SUM of PAHs	--	--	--	--
Centroid of PAHs	--	--	--	--
Copper	50	44	47	28
Cadmium	1.6	1.2	2.3	1.3
Chromium	3.3	1.0	1.6	1.6
Iron	790	840	930	610

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B16

Tissue Residue Concentrations in *N. incisa* from the T + 74 Weeks Field Collection in CLIS (16 Oct 84)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	500	7.9	5.1	3.2
Sum of 178 alkyl homologs	4,800	200	124	33
Fluoranthene	1,410	96	57	27
Benzo(a)pyrene	102	40	46	19
Ethylan	13.6	0	0	0
PCB as A1254	710	510	350	300
SUM of PAHs	16,000	1,660	1,320	580
Centroid of PAHs	208.1	233.9	242.5	246.3
Copper	86	--	--	--
Cadmium	0.6	--	--	--
Chromium	2.5	--	--	--
Iron	680	--	--	--

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.

Table B17

Tissue Residue Concentrations in *N. incisa* from the T + 140 Weeks Field Collection in CLIS (24 Jan 86)*

Chemical Compound	Station				
	CNTR	200E	400E	1000E	REFS
Phenanthrene	7.3	--	4.7	12.0	--
Sum of 178 alkyl homologs	1,070	--	58	390	--
Fluoranthene	300	--	23	78	27
Benzo(a)pyrene	162	--	21	91	23
Ethylan	6.2	--	0	0	0
PCB as A1254	900	--	310	300	160
SUM of PAHs	6,400	--	630	3,000	660
Centroid of PAHs	232.1	--	244.0	244.5	253.7
Copper	83	53	44	46	32
Cadmium	1.8	0.9	0.6	0.8	0.7
Chromium	9.9	4.3	2.4	2.0	2.1
Iron	840	970	1,250	920	970

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic centroid.